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A STUDY OF THE PIGMENTS PRODUCED IN DARKNESS BY CERTAIN GREEN ALGAE

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(WITH FIVE FIGURES)

An observation which has become well established is that certain green plants (*e.g.*, some of the green algae) are able to produce their chlorophyll in the dark. The experimental data characterizing the pigments so produced, however, are indeed meager. It therefore has seemed advisable to reinvestigate the pigments formed by such plants in the dark with the improved spectroscopic methods now available. In the first place there is no conclusive evidence that tells whether or not chlorophyll *b* is formed in darkness. Secondly, it is to be hoped that a study of these rather exceptional cases of chlorophyll formation in the dark may well be a foothold in the study of the general process of pigment development. And thirdly, it is conceivable that spectroscopic differences between "light" and "dark" pigments might be found which could later be correlated with the beginnings of photosynthesis.

This paper is therefore a report of the data so far obtained in a spectroscopical and physiological study of the pigments formed by green algae in the dark.

Although it had previously been noted (*e.g.*, HEINRICHER (**7**), SCHIMPER (**15**), and KLEBS (**8**)), the first unqualified demonstration of the greening by algae in the dark seems to have been made by BEIJERINCK (**3**) in 1890 on a pure culture of *Chlorosphaera limicola*.[1] A later paper by the same worker reported (**4**) that various pure cultures of green algae (including *Chlorella vulgaris, Stichococcus bacillaris,* and *Scenedesmus acutus*) had been grown for several years in absolute darkness. The cultures developed quantities of green cells which again became autotrophic when brought into light. The work of ARTARI (**1**) also demonstrated the greening of algae and lichen gonidia in darkness. ÉTARD and BOUILHAC (**6**) compared spectroscopically

[1] A colored plate shows green cells that had been grown in the dark on a sucrose-peptone gelatin.

the alcoholic extracts of the blue-green alga, Nostoc, grown on sugar solution in darkness with extracts of green leaves. Their methods gave no significant differences between the two. RADAIS (**14**) made a somewhat more detailed spectroscopic study of the alcohol and carbon disulphide extracts of parallel light and dark cultures of *Chlorella vulgaris*. He obtained identical absorption spectra for the extracts of the two cultures. In addition to the "end absorption" below 511 mμ he observed three absorption bands with mean axes at 667, 618, and 577 mμ. His carbon disulphide extract gave bands with mean axes at 679, 659, 625, and 583 mμ. The occurrence of a distinct additional absorption band in the red at 659 mμ for the carbon disulphide extract suggests the presence of the *b* component. It is rather difficult, however, to see why the alcoholic extracts failed to show a similar band. And by analogy to the absorption curves in other solvents (*e.g.*, ZSCHEILE (**25**)) it seems rather surprising that a chlorophyll *b* peak would show up so clearly in this region unless the *b* component were present in an unusually high proportion.

DANGEARD (**5**) cultivated *Scenedesmus acutus* for eight years in darkness and found that it maintained its green color. The algae brought into light at the end of that time showed within five hours a photosynthetic activity (liberation of bubbles of oxygen). Unfortunately DANGEARD's technique of observing photosynthesis was neither rapid enough nor sufficiently quantitative. Considerable pigment changes might have taken place in less than five hours or might still have been going on at the end of that time (*cf.* SEYBOLD, **16**).

While the available data are consistent with the view that the pigments formed in darkness by certain green algae are photosynthetically effective, in the light of more recent knowledge it has seemed advantageous to reinvestigate this problem with the improved methods of pigment study and photosynthesis measurement now available.

Since the completion of the experimental work of this paper, there has come to the author's attention the recent publication of VAN HILLE (**22**). Incidental to his study of the relation between rate of photosynthesis and chlorophyll content this worker studied *Chlorella pyrenoidosa* grown in darkness. He states that methanol extracts of cells grown in light and in darkness showed the same absorption spectra, although he includes no data and apparently worked only at wavelengths greater than those absorbed by the carotenoids. VAN HILLE also found for cells grown in darkness (p. 748) that "The readings per half-hour of photosynthetic measuring are constant from the beginning and show the normal time of induction (SMITH, 1937)." Again no data are included. Although VAN HILLE's findings have been duplicated, the present paper is considered justified by the more detailed and comprehensive data obtained.

Spectroscopy of the pigments

Three species of green algae were used in this study: *Chlorella vulgaris*, *Protococcus* sp., and *Chlorococcum* sp.[2] These were grown in pure culture on the agarized Detmer's solution diluted to $\frac{1}{3}$ as recommended by MEIER (11) in flat one-liter medicine bottles with loose cotton plugs. Bacteriological technique was observed. Three parallel series were used: (1) in darkness with added organic nutrient; (2) in light with added organic nutrient; (3) in light without added organic nutrient. Added organic nutrient consisted of 0.5 per cent. dextrose and 0.2 per cent. peptone. Cultures in light were continuously illuminated by tungsten filament bulbs with Corning Aklo filters to reduce the infra-red. Intensity at the level of the cultures was 65 foot candles as recorded by the Weston photronic cell.

Common methods of extraction employing cold solvents proved ineffective. The use of hot solvents was considered dangerous in view of the labile nature of the pigments. A method has been devised which is a modification of the method used by MUDD and co-workers (13) for the extraction of labile bacterial antigens. A suspension of algal cells, washed off the agar surface, is placed in a metal ball-mill[3] and rapidly frozen around the inside rim by rotating the mill in a $-80°$ C. bath (dry ice in alcohol). Freezing is completed in less than one minute. The ball-mill is then quickly attached to a high vacuum line in series with a low temperature condenser held at $-80°$ C. The ice is thus rapidly sublimed off and drying is complete in 12 to 16 hours. The mill is then returned to the low temperature bath and two steel balls inserted. After an hour and a half grinding the bath is removed, 90 per cent. acetone introduced into the ball-mill, and grinding continued for another hour. The acetone extract is removed and the pigments transferred to ethyl ether. (Anesthesia ether, Malinckrodt, containing 2.5 per cent. alcohol was used throughout because of its very low peroxide content.)

Unfortunately the above procedure is not quantitative in that not all of the algal cells are crushed and extracted. Conditions are nearly optimal, however, for the preservation of labile and easily oxidizable cellular materials such as the chlorophylls and carotenoids.

Spectral absorption curves on the ether solutions have been determined, using the photoelectric spectrophotometer previously described by MILLER (12) and observing the precautions which he suggested. At some time during each run the calibration curve for prism setting was checked (± 2 Å)

[2] Identified by Professor FELIX MAINX of the German University at Prague. Obtained through the courtesy of Dr. C. E. SKINNER by whom they had been isolated from soil.

[3] Kindly provided by the Department of Veterinary Science, the University of Minnesota.

against the 6402 Å neon line. The slit-widths used varied from 0.04 mm. at 4000 Å and 0.02 mm. at 4200 Å to about 0.002 mm. at 6000–6800 Å. In the absorption curves to be presented intensity of absorption is designated merely as $\log \frac{I_o}{I_x}$ according to Beer's Law, since concentrations are not known. The wavelength axes are in Ångström units.

A method previously used by STRAIN (20) has been followed in order to separate into two groups the chlorophyll and carotenoid pigments. This is demonstrated by the curves of figure 1 for the extracted pigments from

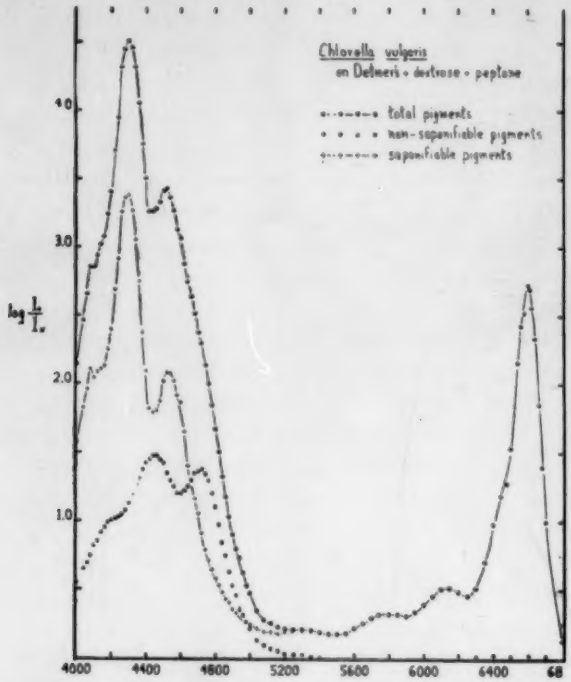

FIG. 1. Absorption spectra of pigments in ethyl ether + 2.5 per cent. alcohol extracted from *Chlorella vulgaris* grown in darkness. Curves refer to equal aliquots of the original extract. For further explanation see text.

Chlorella vulgaris grown in darkness. The upper curve describes the light absorption of the total pigments. If an equal or known aliquot of this ether solution is saponified and the non-saponifiable pigments taken up in ether, the lower curve is obtained. This may be considered the absorption curve for the carotenoid pigments. If the lower curve is now subtracted from the upper curve, point-by-point in the original data, the intermediate curve is obtained. This may be considered the absorption curve of the saponifiable pigments, *i.e.*, of the chlorophylls.

The method of course depends upon the assumption that the saponification removes all the chlorophylls but destroys none of the carotenoids. In my hands the common cold saponification has failed to remove all the chlorophylls, as shown by residual light absorption at 6400–6600 Å. A hot saponification has been required.[4] Comparison of hot and cold saponification on equal aliquots of the pigments of *Chlorococcum* sp. grown in the dark is given in figure 2. Intensity of absorption is significantly higher after the

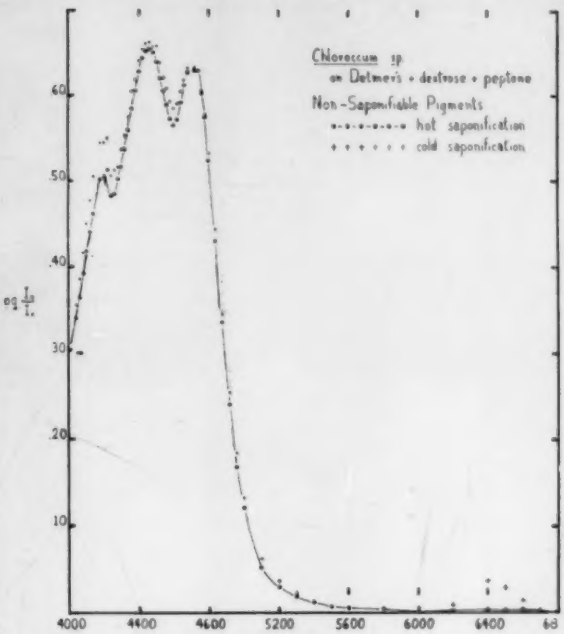

FIG. 2. Absorption spectra of the pigments remaining after hot and after cold saponifications. Pigments originally extracted from *Chlorococcum* sp. grown in darkness.

cold saponification only in those spectral regions where the chlorophylls show high absorption.

Figure 3 shows the absorption curves for the total pigments extracted from *Protococcus* sp. grown under three different conditions. For two of these curves all values of $\log \frac{I_o}{I_x}$ have been multiplied by a factor so chosen

[4] To 4 ml. of 30 per cent. KOH in methanol refluxing over a water bath, 5–10 ml. of the ether solution are added and refluxing continued for 3 minutes. The total solution is then poured into separatory funnel containing ice water and about 10 ml. of ether. The resulting ether solution of carotenoids is washed 5 times with water, separated, and made up to the desired volume. For cold saponification the same time, quantities, and reagents were used and the entire procedure carried out in a separatory funnel.

as to give identical values for the intensity of absorption at 6600 Å on all curves. This merely brings the curves together for ease of comparison. The corresponding absorption curves for the chlorophyll pigments, obtained as described above, are presented in figure 4. Agreement is rather striking, especially through the longer wavelengths of the spectrum. Noticeable variations occur in the height of the bands in the blue: the 4520 Å peak of chlorophyll *b* and the 4100 Å and 4300 Å bands principally due to chlorophyll *a*. These variations may be accounted for in part by variations in the ratio of *a* to *b*.

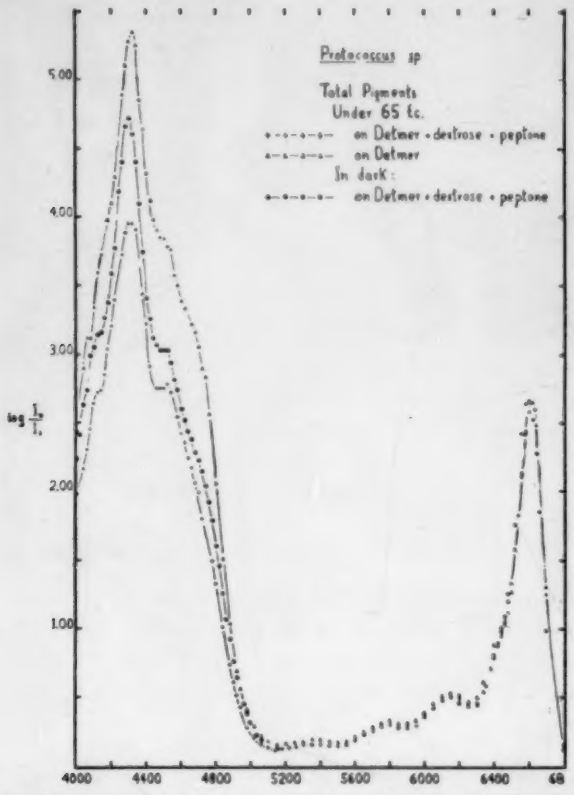

FIG. 3. Absorption spectra of the total pigments extracted from *Protococcus* sp. grown under three different conditions.

Comparison of the chlorophyll curves has been made with the absorption curves of the isolated components obtained by ZSCHEILE (**25**) on the same type of instrument. In general the curves of figure 4 (for ether + 2.5 per cent. alcohol) show a displacement of the peaks toward the red of about 10 to 25 Å. Because of this shift a quantitative analysis of the curves for percentages of the components *a* and *b* by the method of ZSCHEILE (**25**), and

based on his curves for the chlorophylls in ether solution, is impossible. Only anomalous results can be obtained.

The presence of the well-defined 4520 Å band in all the curves of figure 4 as well as in the intermediate curve of figure 1 makes it clear that chlorophyll *b* (or something very similar) is formed in the dark by these cultures of Protococcus and *Chlorella vulgaris*. And inspection of the curves of figure 4 shows that in Protococcus the chlorophyll *a*: *b* ratio is at least of the same

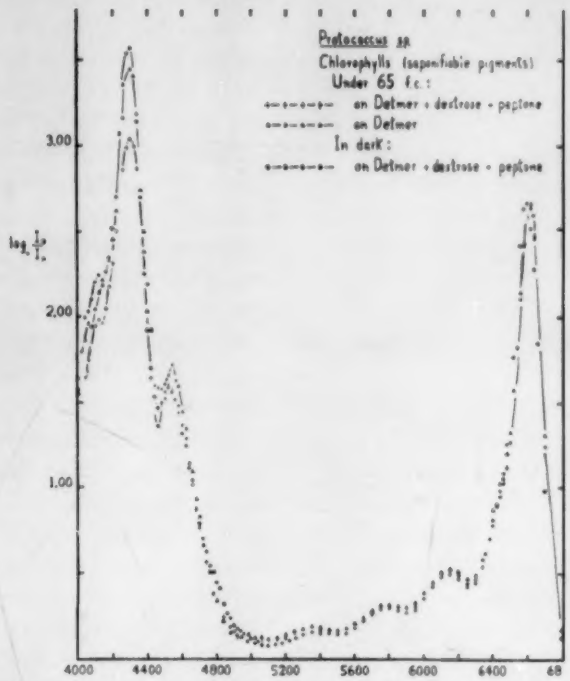

FIG. 4. Absorption spectra of the chlorophylls produced by *Protococcus* sp. grown under three different conditions.

order of magnitude for cells grown in light or in darkness. The formation of chlorophyll *b* in the dark has been confirmed by the appearance of distinct and characteristic chlorophyll *b* zones on sucrose chromatograms prepared according to the method of SEYBOLD and EGLE (17). Comparison of the curves of figures 3 and 4 indicates that the carotenoid: chlorophyll ratio varies considerably under the different conditions of growth. More detailed examination of the carotenoids should be made to determine whether these variations are qualitative or merely quantitative in nature. Further chromatographic work planned for the study of the yellow pigments has not as yet been carried out.

Development of photosynthetic activity

It was advisable at this point to consider the development of photosynthetic activity in algal cells which had previously grown only in darkness. Essentially this involved a comparison of the induction periods of cells grown in darkness and in light. Measurements were made by the familiar Warburg technique, using one illuminated flat-bottom vessel and a non-illuminated thermobarometric control. The volume of the experimental flask was 14.22 ml. to the level of Brodie fluid in the manometer, as calibrated with mercury. When used as described below 1.0 mm. increase in pressure corresponds to an oxygen evolution of 0.56 cu. mm.

The constant-temperature bath was held at 26° C. $\pm$ 0.01° as checked by a Beckmann thermometer. Light was provided by an optical system mounted horizontally beneath the bath. The light from a projection bulb, suitably condensed by two lenses, was reflected vertically up through the glass bottom of the bath by a concave mirror. The area of the light beam in cross-section at the level of the experimental flask was large enough so that the flask was always illuminated during the three-cm. amplitude of its shaking cycle. This arrangement was designed to give very high light intensities, since previous work [WARBURG (**24**), SMITH (**19**), MCALISTER (**9**)] indicates that the induction period is longer, and therefore more easily measurable, under higher intensities. At very high light intensities, however, (about 5,000–20,000 f.c.) an inhibition of photosynthesis was observed. A study of this inhibition is to be reported elsewhere. For measurement of the induction period an intensity of 2,200 f.c. was used.

Preliminary results indicated that more consistent results could be obtained for cells grown in liquid culture than for those grown on solid media as in the pigment study. It was also found that *Chlorella vulgaris* was much better adapted for this part of the study than the other algae available. *Protococcus* sp. could not be centrifuged out cleanly and showed such low rates of photosynthesis that it was more difficult to work with. Cultures were grown in 500-ml. Erlenmeyers with a current of air bubbled through. To obtain darkness, flasks were wrapped in photographic light-proof paper and kept in covered iron pails. Cultures grown in light were placed uniformly around a 5.5-cm. water jacket surrounding a 300-watt bulb. The intensity at the illuminated side of the flasks was about 450 f.c. Sterile precautions were observed only when glucose was added to the nutrient solution, although microscopic tests for purity were made in all cases.

Measurements were made with the cells suspended in the potassium carbonate-bicarbonate buffer corresponding to the sodium carbonate-bicarbonate buffer no. 9 of WARBURG (**23**) (0.015 M K_2CO_3, 0.085 M $KHCO_3$). Immediately before an experimental run the cells were centrifuged out of the nutrient solution, taken up in the buffer, and centrifuged out again in

a graduated tube. To the packed cells fresh buffer was added to give a suspension in which 1.0 ml. contained 0.05 ml. of cells. The suspension was then kept in the dark and aliquot portions withdrawn in the dark by an automatic pipette. When cells were grown in darkness, all further operations except the brief reading of cell volume were performed in the dark.

One ml. of cell suspension was added to 7.2 ml. of buffer in the experimental flask. The flask and manometer were placed in position without illumination and about five minutes allowed for adjustment to equilibrium. Four or five consecutive five-minute readings were taken to establish the course of respiration. Light was then turned on for ½ minute. Respiration in the succeeding dark period was again established by at least four readings. This procedure was repeated with light exposures of 1, 1.5, 2, 3, and 4 minutes, using a fresh batch of cells for each exposure. The displacement between the respiration curves before and after the light exposure is a measure of the amount of photosynthesis which took place. This graphical method is illustrated by the upper curves of figure 5. It is an "integrational" method necessitated by the lag in the Warburg instrument.[5]

The method is of course limited to the accuracy of extrapolation of the respiration curves. Occasionally a deviation in the rates of respiration before and after illumination was observed, but never of more than about 5 per cent. It is recognized that such a variation introduces a possible source of error in the extrapolation. Several preliminary experiments seemed to indicate that more consistent results were obtained when a fresh aliquot of cells was used for each time interval and when 0.5 per cent. dextrose was added to the stock suspension kept in the dark. These conditions were therefore observed throughout.

The induction curves obtained for *Chlorella vulgaris* as described above are shown in figure 5. The close coincidence of the curves for cells grown with 0.5 per cent. dextrose, with and without light, clearly answers the problem of this investigation. *The pigments produced by this green alga in darkness are adequate for the process of photosynthesis.* Cells grown in the light without dextrose (upper curve) attain a somewhat higher photosynthetic rate and seem to have a somewhat longer induction period than cells grown with dextrose. The *induction loss,* however [extrapolated intercept on the photosynthesis axis, *cf.* McALISTER (**9, 10**)], is practically identical for all three lots of cells.

In considering the above data as a further description of the induction phenomenon, a possible limitation of the integrational method must be taken into account. McALISTER (**10**) has demonstrated that after high rates of photosynthesis there is an appreciable "pick-up" of CO_2 after the plant is

[5] This is essentially the same method previously used by WARBURG (**24**), VAN DER PAAUW (**21**), and SMITH (**19**).

darkened. Making the reasonable assumption that there is also a continued evolution of O_2 by a plant on darkening, comparable to the "pick-up" of CO_2, he has suggested that there may be an inherent error in the integrational method used here. What is probably measured for each period of illumination is the actual amount of photosynthesis during illumination plus the "pick-up" (in this case the extra evolution of oxygen) on darkening. Each point on the induction curve is therefore too high by an amount equal to the "dark pick-up" involved in its determination. So an induction curve measured by the integrational method is probably somewhat in error in regard to its shape and *induction loss*. From the characteristics of the "dark pick-up," however, it seems unlikely that the indicated length of the induction period will be appreciably in error.

The papers of McAlister (**10**) and Aufdemgarten (**2**) have shown that the induction period is a function of the preceding dark rest when photosynthesis is measured by the rate of CO_2 uptake. Aufdemgarten (**2**) states that the induction period in Stichococcus is lengthened by increasing dark rests of up to fifteen minutes, beyond which no further effect was noted. McAlister's (**10**) more comprehensive data for wheat show a rapid increase in *induction loss* with dark rests increasing up to about one minute, followed by a more slowly progressing effect up to about eight hours. These papers had not yet appeared when the experimental work was done. No attempt was made to control the preceding dark rest, which varied from about two to eight hours during each run for cells grown in light. The experimental points, however, were not obtained in any definite order and any appreciable change in the true induction period during this time would hardly have permitted the points for the two, three, and four minutes illumination to fall on a straight line. Of course, the final straight line may not be truly constant in slope. Indeed, in continuous light all three batches of cells showed photosynthetic rates increasing slowly with time and always slightly higher than the final rates indicated by the curves in figure 5. But following the usual interpretation, the induction period is clearly over somewhere between one and two minutes of illumination. Certainly dark rests of more than two hours could have had little if any additional effect on the characteristics of the induction period in *Chlorella vulgaris*.

It is also of interest to compare the induction curves (fig. 5) with those obtained by other workers using the same method, which is based on the rate of evolution of oxygen. Smith (**19**), using a preceding 30-minute dark rest, found for Cabomba induction periods of about two to five minutes depending on the light intensity and CO_2 concentration. Van der Paauw's (**21**) curves for Hormidium show an induction of $1\frac{1}{2}$ minutes at 26° C. (length of dark rest not stated). Warburg (**24**) observed for *Chlorella vulgaris* an induction period of $1\frac{1}{2}$ minutes after a five-minute dark rest. His conditions

of light intensity, CO_2 concentration, and temperature were almost identical with those maintained in the present study. The induction period obtained by WARBURG after a five-minute dark rest is identical with that shown in figure 5 for the same organism after an infinitely long dark rest. This is also in line with WARBURG's data for one- to five-minute periods of intermittent light.

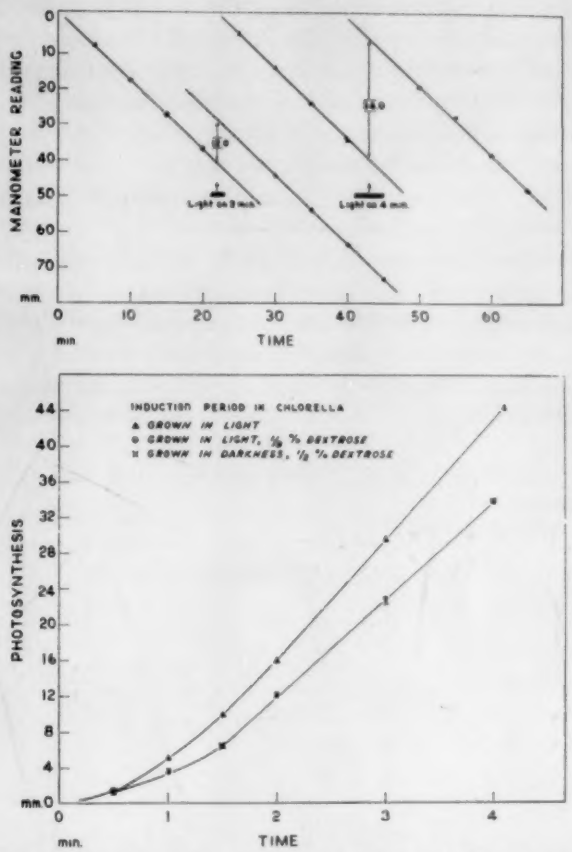

FIG. 5. The induction period in *Chlorella vulgaris*. Ordinates are readings from the Warburg manometers in mm.; multiply by 0.56 to obtain cu. mm. of O_2. The upper set of curves describes the method by which each point in the lower set of induction curves was obtained. For further explanation see text.

Any detailed study of the effect of the preceding dark rest on the induction period was not a planned part of this investigation, and the results are certainly not critical in this respect. However, after comparison of the data of WARBURG and of this paper on one hand with those of MCALISTER or of AUFDEMGARTEN on the other, it seems that the induction in O_2 production

and the induction in CO_2 uptake are not equally dependent upon the preceding dark rest, and are therefore probably dissimilar in other respects.

Summary

1. The pigments produced by *Protococcus* sp. and *Chlorella vulgaris* in darkness have been studied with a photoelectric spectrophotometer and compared with the pigments produced in light. For this purpose a method of extraction not previously used in pigment work has been described.

2. No significant qualitative differences have been found between the chlorophyll pigments produced in light and in darkness.

3. The induction periods for cells grown in light and in darkness have been studied by the WARBURG technique for a strain of *Chlorella vulgaris*. Only slight differences have been found between the induction curves of cells grown in darkness and in light.

4. The pigments produced by this strain of *Chlorella vulgaris* in darkness are adequate for the process of photosynthesis.

5. From a comparison of the data obtained with other data in the literature on the induction period it has been suggested that there is a dissimilarity between the induction in O_2 evolution and the induction in CO_2 uptake.

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LITERATURE CITED

1. ARTARI, A. Über die Bildung des Chlorophylls durch grüne Algen. Ber. d. bot. Ges. **20**: 201–207. 1902.
2. AUFDEMGARTEN, H. Zur Kenntnis der sogenannten Induktionsvorgänge bei der Kohlensäureassimilation. Planta **29**: 643–678. 1939.
3. BEIJERINCK, M. W. Culturversuche mit Zoochlorellen, Lichengonidien und anderen niederen Algen. Bot. Zeit. **48**: 724–771; 781–785. 1890.
4. ————————. Notiz über *Pleurococcus vulgaris*. Cent. f. Bakt. II **4**: 785–787. 1898.
5. DANGEARD, A. P. Observations sur une algue cultiveé à l'obscurité depuis huit ans. Compt. Rend. Acad. Sci. (Paris) **172**: 254–260. 1921.
6. ÉTARD, A. and BOUILHAC, M. Presence des chlorophylles dans un Nostoc cultivé à l'abri de la lumiere. Compt. Rend. Acad. Sci. (Paris) **127**: 119–121. 1898.
7. HEINRICHER, E. Zur Kenntnis der Algengattung Sphaeroplea. Ber. d. bot. Ges. **1**: 433–450. 1883.

8. KLEBS, G. Beiträge zur Physiologie der Pflanzenzelle. Ber. d. bot. Ges. **5**: 181–188. 1887.
9. MCALISTER, E. D. Time course of photosynthesis for a higher plant. Smithsonian Misc. Coll. **95**: no. 24. 1937.
10. ————. The chlorophyll-carbon dioxide ratio during photosynthesis. Jour. Gen. Physiol. **22**: 613–636. 1939.
11. MEIER, F. Effects of intensities and wavelengths of light on unicellular green algae. Smithsonian Misc. Coll. **92**: no. 6. 1934.
12. MILLER, E. S. A precise method, with detailed calibration, for the determination of absorption coefficients; the quantitative measurement of the visible and ultra-violet absorption spectra of alpha carotene, beta carotene, and lycopene. Plant Physiol. **12**: 667–684. 1937.
13. MUDD, S., SHAW, C. H., CZARNETZKY, E. J., and FLOSDORF, E. W. A low-temperature ball-mill for the liberation of labile cellular products. Jour. Immunol. **32**: 483–489. 1937.
14. RADAIS, M. Sur la culture pure d'une algue verte; formation de chlorophyll à l'obscurité. Compt. Rend. Acad. Sci. (Paris) **130**: 793–796. 1900.
15. SCHIMPER, A. F. W. Untersuchungen über die Chlorophyllkörper und die ihnen homologen Gebilde. Jahrb. wiss. Bot. **16**: 1–247. 1884.
16. SEYBOLD, A., and EGLE, K. Lichtfeld und Blattfarbstoffe. II. Planta **28**: 87. 1938.
17. ————, and ————. Zur chromatographischen Methode der Blattpigmente. Planta **29**: 114–119. 1938.
18. SMITH, E. L. The influence of light and carbon dioxide on photosynthesis. Jour. Gen. Physiol. **20**: 807–830. 1937.
19. ————. The induction period in photosynthesis. Jour. Gen. Physiol. **21**: 151–163. 1937.
20. STRAIN, H. H. Leaf xanthophylls. Carnegie Inst. Washington. Pub. no. 490. 1938.
21. VAN DER PAAUW, F. The indirect action of external factors on photosynthesis. Pp. 595–598. Amsterdam, 1932.
22. VAN HILLE, J. C. The quantitative relation between the rate of photosynthesis and chlorophyll content in *Chlorella pyrenoidosa*. Rec. trav. bot. néerl. **35**: 682–757. 1938.
23. WARBURG, O. Über die Geschwindigkeit der photochemischen Kohlensäurezersetzung in lebenden Zellen. Biochem. Zeitschr. **100**: 230–270. 1919.
24. ————. Über die Geschwindigkeit der photochemischen Kohlensäurezersetzung in lebenden Zellen. II. Biochem. Zeitschr. **103**: 188–217. 1920.

25. ZSCHEILE, F. P., Jr. An improved method for the purification of the chlorophylls A and B; quantitative measurement of their absorption spectra; evidence for the existence of a third component of chlorophyll. Bot. Gaz. 95: 529–562. 1934.

USE OF TENSIOMETERS IN REGULATING WATERING IN FOREST NURSERIES[1]

J. H. Stoeckeler and Einar Aamodt

(WITH SIX FIGURES)

Introduction

Soil moisture is one of the most important factors influencing physiological processes, such as growth of plants, their development as regards root and top, effect on drought resistance, frost hardiness, and dormancy. These physiological effects have a special importance in large-scale forest nursery production where the maintenance of a proper water supply in the soil is one of the controlling factors in the production of high-quality planting material. Methods of determining soil moisture are still susceptible of improvement and any advances made in this field are of practical significance.

The regulation of watering in forest nurseries has always been a problem, even in those nurseries equipped with overhead irrigation systems. Some of the difficulty has come about because of differences in soil, species, topography, windbreak protection, shade, age class, and relative density at which the stock is grown. It has been observed that first-year conifer seedlings need light but frequent watering, whereas older stock needs fewer but heavier irrigations. The smaller seedlings require more frequent watering because of their limited depth of rooting, and because their thin cortex is not as good an insulator against sun injury as is the thicker bark of seedlings two or more years old. The sandier and the more exposed parts of the nursery need more frequent irrigation than the rest of the nursery. Seedlings under half shade require less than those without shade.

Although irrigation will be based largely upon the experience of the nurseryman, his judgment will be aided materially by some instrumental method of measuring the moisture content of the soil.

The chief disadvantages of many of the available instruments have been that they are difficult to construct and operate or too expensive to purchase in sufficient numbers to get adequate coverage in the nursery. Oven-drying of soil samples is considered impracticable owing to the time involved in taking samples and also to the fact that results cannot be obtained until about 24 hours after sampling. There are, of course, several other methods, including the alcohol-burning method described by Bouyoucos (**1**) and auto-irrigators or soil points (**6**), which provide a means of getting results in an

[1] Contribution from Lake States Forest Experiment Station, University Farm, St. Paul, Minnesota. Maintained by the U. S. Department of Agriculture, Forest Service, in cooperation with Division of Forestry, University of Minnesota.

hour or less. These have been proved to be of practical value in forest nurseries, but are relatively time consuming.

One of the instruments developed in recent years which shows considerable promise in gauging moisture conditions in soils is the "tensiometer."[2] This device measures capillary pull of soils at various moisture contents. The so-called capillary pull, in simple terminology, represents the amount of force with which the wedges or lenses of capillary water between soil particles are held against extraction. In the usual type of tensiometer this force is expressed in centimeters or millimeters of mercury and is determined by attaching to a mercury manometer a fine-textured porous clay pot filled with boiled distilled water. This pot is buried in the soil at the desired depth and the rise in the mercury column is observed. The drier the soil, the higher will be the mercury lift observed on the manometer. If a series of readings is taken over a range of moisture conditions, it is possible to obtain a calibration curve for any specific soil at a given degree of compaction that shows the equivalent of a certain moisture content expressed in centimeters of mercury. Where a more rugged and durable type of instrument is needed, an industrial vacuum gauge may be substituted for the more fragile mercury manometer.

The one serious objection to tensiometers is that they will record a maximum pull of about 55 to 65 centimeters of mercury, and it has been shown for some of the fine-textured soils, such as clays, or clay loams, that this lift represents only about 70 to 85 per cent. of the moisture range in which plants will survive. Such heavy soils, however, are not ordinarily used for conifer nurseries, and lifts of 0 to 50 cm. of mercury represent practically the entire range of moisture conditions in which small conifers can survive and develop properly in sandy soils.

Tensiometers have been used on an experimental basis by a number of investigators. KORNEV (**5**) in 1921 devised one of the first tensiometers by making use of the method already described, although LIVINGSTON (**6**) mentions that similar experiments had been conducted as early as 1908 at the Desert Laboratory near Tuscon, Arizona.

LYNDE and DUPRÉ (**7**) devised apparatus in 1913 to study the capillary lift of soils at pressures ranging from 1.37 to 0.74 atmospheres, and concluded that capillary lift was limited by pressure of the atmosphere and that maximum lifts under one atmosphere of pressure could not exceed 10 meters, or 34 feet.

HEATH (**2**), in a rather intensive test, made up 30 of these instruments,

[2] In this paper the terms "tensiometer," "tonometer," and "moisture meter" are used synonymously. The term "tensiometer," recommended by L. A. RICHARDS, is undoubtedly the most descriptive and accurate in a scientific sense, but "moisture meter" is probably the better term for the layman.

varied the moisture content of the soil, and reported a good correlation of the manometer readings with the amount of water added.

HECK (**3**), with a modification of the tensiometer built by HEATH, successfully used such instruments as a guide in determining when it was necessary to irrigate sugar cane in Hawaii. He reports that when the soil became dry enough to lift the mercury column 25 or 30 centimeters, active growth practically ceased and it was necessary to apply irrigation water to bring the soil moisture back to an optimum for continued rapid plant growth.

In a technical discussion of the relation of physical properties of soil to capillary behavior, HAINES (**4**) shows that a marked increase in pressure deficiency occurs when the soil is at 80 per cent. or less of saturation, and climbs sharply to a point at 20 per cent. of saturation, from whence the curves flatten off. His work has a bearing on the use and calibration of tonometers, for he shows that the pressure-deficiency curve for rising moisture does not coincide entirely with that for falling moisture. This phenomenon is referred to as "hysteresis" and is further discussed in detail by SMITH (**13**).

This discovery has a practical bearing on the use of tensiometers in field setups because moisture content in the field, especially in the surface layers of soil, is generally slowly dropping, except during periods of replenishment by rainfall or irrigation. The inference is that in calibration of instruments for field use, the meters should be calibrated in a soil changing from a moist to a dry state.

RICHARDS and GARDNER (**9**) describe the underlying principles of indicating, recording, and differential tensiometers and mention the fact that temperature changes require a correction for capillary tension readings obtained with such instruments. In an earlier paper (**8**), RICHARDS discusses other factors influencing capillary potential and illustrates how the degree of packing of a soil affects the curvature of the water wedges between the soil particles, any change of which results in a different potential.

RICHARDS and NEAL (**10**) in a further study of tensiometers found considerably greater fluctuation in readings for shallow depths of 6 to 12 inches than at depths of 24 and 36 inches; this indicated a rapid response to rainfall and evaporation in the surface layer of soil. They also noted a diurnal fluctuation in capillary tension in the surface soil. This was explained on the basis of the varying evaporation rate of moisture from the soil and also by the possible effect of soil temperature and of the water in the tensiometer system.

ROGERS (**11**) devised a tensiometer that recorded capillary pulls of over 60 centimeters and incorporated an anti-freezing device that permitted the use of such instruments earlier in spring and later in fall than would ordi-

narily be possible. He gives calibration curves for several soils that indicate a striking difference, depending on texture.

Apparatus

Design of the Lake States moisture meter

Because of the necessity for getting more accurate control of soil moisture in forest nurseries in the Lake States, experiments were started in the spring of 1937, and carried out through 1938 and 1939, to determine the feasibility of various methods of determining soil moisture. Among the methods and devices tried were oven-drying, soil points, auto-irrigators, and tensiometers (**14**). The present paper will be confined to the presentation of data and experience obtained in constructing 42 tensiometers and from studies of several types of these instruments and their practical use in forest nurseries in growing drought-hardy and frost-resistant stock.

The first model (fig. 1) was patterned very closely after the instrument

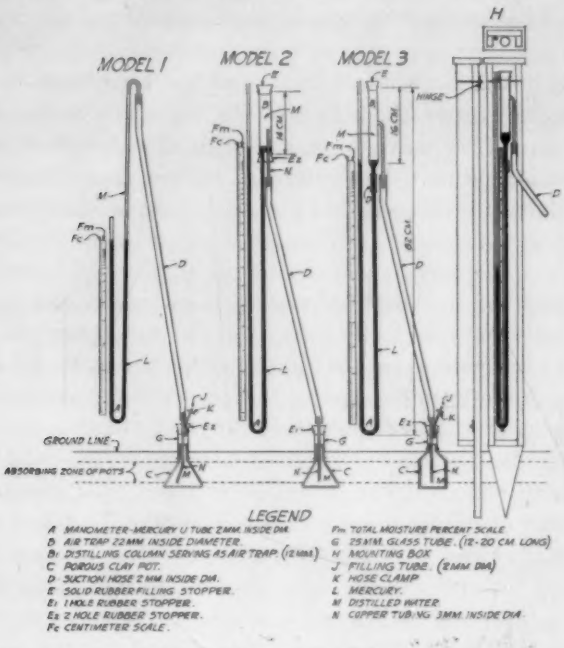
MODEL 1
MODEL 2
MODEL 3
H
HINGE
GROUND LINE
ABSORBING ZONE OF POTS
LEGEND
A MANOMETER-MERCURY U TUBE 2MM. INSIDE DIA.
B AIR TRAP 22 MM. INSIDE DIAMETER.
B1 DISTILLING COLUMN SERVING AS AIR TRAP. (12 MM.)
C POROUS CLAY POT.
D SUCTION HOSE 2 MM. INSIDE DIA.
E SOLID RUBBER FILLING STOPPER.
E1 1 HOLE RUBBER STOPPER.
E2 2 HOLE RUBBER STOPPER.
Fc CENTIMETER SCALE.
Fm TOTAL MOISTURE PERCENT SCALE.
G 25 MM. GLASS TUBE. (12-20 CM. LONG)
H MOUNTING BOX.
J FILLING TUBE. (2 MM. DIA)
K HOSE CLAMP.
L MERCURY.
M DISTILLED WATER.
N COPPER TUBING 3 MM. INSIDE DIA.

FIG. 1. Details of construction of several types of tensiometers.

designed by HECK (**3**). It differed mainly in that a LIVINGSTON auto-irrigator pot having a sloping porous side wall with an area of 100 cm.^{2} was substituted for the elongated porous candle. A number of moisture meters of this type were made up, but did not prove entirely satisfactory owing to

the difficulty of preventing air from getting into the irrigator pot and eventually lodging in or near the top of the water tube connected with the mercury manometer. When a small amount of air lodged in the water column, the instrument did not function effectively.

One of the model 1 (fig. 1) tonometers, however, gave good results over a 2-month period in a bed of first-year red pine in the loamy fine sand soil at Hugo Sauer Nursery, Rhinelander, Wisconsin, in 1937. This instrument functioned successfully without addition of any water to the pot during the 2-month period; it must be admitted, however, that it was not subjected to a critical test because of favorable moisture conditions. The maximum mercury depression observed with this instrument was 30 centimeters.

Owing to the difficulty caused by air getting into the pot and water column, the instrument was redesigned and fitted with a device used for the dual purpose of adding water and trapping air. This device, designated as an air trap, consisted of a 25-mm. glass tube 14 cm. long (fig. 1, model 2) and was fitted at the top of the instrument. Both the mercury and water tubes were brought in at the bottom of the tube through a 2-hole rubber stopper carefully cemented into place to prevent any leakage of air. The water tube was carried up inside the air trap to a height of about 6 cm. Mercury was first placed in the U-tube in an amount sufficient to bring it to a height of about 0.5 cm. above the flat bottom of the air trap. Boiled distilled water was then run into the open upper end of the system in a very fine stream until the pot, suction hose, and air trap had been completely filled. A smooth-fitting rubber stopper (E) was then fitted tightly into the top of the air trap.

This design considerably simplified the addition of distilled water and the removal of air from the instrument. It also provided a storage reservoir for mercury and permitted the use of a shorter manometer than in moisture meters of the type illustrated by model 1, when due consideration is given to the effective length of the moisture percentage scale.

Variations in temperatures may affect the manometer readings. It thus appeared desirable to reduce the amount of water in the system above the ground line, and a smaller-bore air trap, indicated as item B_1 in model 3, was substituted. This piece had the further advantage that tight connections were more readily obtained. In the third model a larger porous clay jug of finer texture than the LIVINGSTON auto-irrigator pot was used. This Red Wing[3] pot was thick-walled (5 mm.) and had an absorbing surface of 180 cm.^{2} on the vertical side wall, which was 24.5 cm. in circumference and 7.35 cm. high. The bottom and the sloping walls at the top of the cup were given a triple coat of waterproof varnish, so as to waterproof them and leave only the vertical wall as the porous element through which water moved.

[3] These consisted merely of unglazed Minnesota-Michigan souvenir jugs of "flowerpot clay" manufactured by the Red Wing Pottery Co., Red Wing, Minnesota.

This type of jug, in spite of its finer texture, gave more rapid reaction than did the LIVINGSTON cup and was less subject to entrance of air through the porous side walls. It was necessary, however, to test each individual pot by placing it in water and applying air pressure. Any pots with serious flaws, indicated by a stream of bubbles coming from the defective spot, were discarded. Pots with only minor flaws were made serviceable by putting a drop of DE KHOTINSKY cement on the leaky spot. Besides the vertical-walled Red Wing pot, several other types of porous cups, including "alundum" filters and spout tensiometer cups,[4] were tried. The latter type proved quite satisfactory but did not react as quickly as did the Red Wing jug because of the more limited absorbing surface. No doubt the smaller cup is an ideal type of cup for field conditions where fine-textured soils of undisturbed structure are being studied and where readings of over 40 cm. of mercury may be frequently encountered; for lighter soils adapted to growing of conifers, however, the Red Wing pot is quite satisfactory and costs only a fraction of the price charged for the more expensive clay cups or filters.

With the type of instrument shown in figure 1, model 3, any air in the system can readily be seen either in the glass neck (G) of the pot, or in the top of the air trap (B_1). To remove air it is necessary only to remove the stopper E from the air trap, release clamp K slightly, and pour distilled water into the top of the system until all air is removed. The clamp is reset and the stopper inserted slowly to prevent pushing mercury out of the high open end of the mercury manometer. After refilling with water, the mercury will gradually pull down till it is again in equilibrium. This will take from 1 to 20 hours, depending on how dry the soil is. The time required to attain equilibrium in a fairly dry soil can be shortened to a few hours, or less, by applying air pressure to the open end of the U-tube and forcing the mercury down to about the 60-cm. mark before filling the air trap with water and inserting the rubber stopper.

To prevent air leaks it is necessary to coat all joints with waterproof varnish before fitting them together and to treat them again after the first application has dried. Tight joints between the 25-mm. glass tube (G) and the porous pot were obtained by heating them gently in a Bunsen burner or alcohol blow torch and sealing them together with DE KHOTINSKY cement.

It should be mentioned here that placing of mercury in the model 2 and 3 tensiometers was easily accomplished by removing stopper E, running two pieces of thin copper wire down each side of the manometer, and pouring the mercury into the air trap, meanwhile jiggling the wires up and down.

[4] Used extensively by L. A. RICHARDS and manufactured by General Ceramics Co., Keasbey, N. J. These have an absorbing area 6.4 cm. high and are slightly tapered from 3.1 cm. diameter at top to 2.3 cm. diameter at bottom. The total absorbing area is about 57 cm.^{2}

When all traces of trapped air in the mercury had disappeared, the wires were removed and the system was then filled with water.

Calibration of the moisture meter

All moisture-measuring devices that work on the "suction pull" principle must be calibrated for each soil and generally for each depth, since the mercury pull obtained varies according to the texture of the soil particles. Fine soil particles such as silt and clay create a greater pull at a given moisture content than do coarser materials such as sands.

Two different methods of calibrating the moisture meters were tried. In one method the calibration was done directly in the nursery beds by setting up the instrument with a centimeter scale attached and with the porous cup so buried that the absorbing surface was at a depth between 3 inches and 6 inches. Soil samples were then taken in moisture cans over a period of 4 to 6 weeks by means of a smooth brass sampling tube one-half inch in diameter. Three or four cores were taken within a radius of one or two feet of the pot, with only the portion of the core from the 3- to 6-inch zone being retained for determination of the moisture content by oven-drying. All holes made by the sampling tube were filled with soil from the immediate vicinity, repacked with a wooden dowel, and the surface smoothed so as to avoid leaving either a depression or knoll. The depression of mercury in millimeters below the true equilibrium point[5] was recorded and the values obtained were plotted on coordinate paper and an averaging curve drawn. Then a scale that showed total moisture content and indicated the wilting coefficient[6] of the

[5] When water is contained in the system of the tensiometer shown in figure 1, the mercury in the open end of the manometer will drop from 5 to 7 cm. below the level of the mercury in the air trap. This is due to the hydrostatic head caused by the water in the air trap and in the rubber tube that connects the manometer with the porous pot C. The simplest way to determine the true zero or equilibrium point is to decide how far the center of the absorbing surface of the porous pot will be below the bottom of the mercury U-tube in the actual field setup; then in the laboratory, set up the instrument with the porous cup the same distance below the bottom of the manometer and submerged in water to half the depth of the absorbing surface. In 10 to 20 minutes the mercury will come to the true zero point. This is marked on the box in which the manometer is housed and all readings during calibration are expressed in millimeters of mercury below that point.

[6] Determined indirectly by formula; wilting coefficient $= \frac{\text{moisture equivalent}}{1.84}$. For all practical purposes this value for sandy soils is so close to the actual wilting percentage that there is no reason for attempting to determine it by any other method. The value derived from $\frac{\text{moisture equivalent}}{2.0}$ appears to lie closer to the true wilting coefficient but it is felt that the difference in values derived by the two ratios would represent a certain desirable margin of safety in regulating moisture in forest nurseries. Therefore the first ratio mentioned is preferred.

soil at that location was substituted for the centimeter scale. The scale was made of aluminum embossing tape about one centimeter wide and 70 centimeters long. The figures and cross lines were drawn on the tape with India ink, allowed to dry, and then given several coats of waterproof varnish. The scale was held with tacks to the back of the wooden mounting box. The latter was painted white to reduce the solar heating of the mercury and water in the system, which has a tendency to cause errors in the readings. The portion of the moisture scale that was within 1 per cent. of the wilting coefficient was painted red to indicate a dangerously low moisture content.

The importance of degree of packing of soil and the necessity of undisturbed structure in calibration of tensiometers have been pointed out by several investigators, but in the case of forest nurseries the top 6 to 10 inches of soil have no definite structure because of frequent plowing, rototilling, and digging of trees. In using tensiometers in forest nurseries it is, however, considered important to obtain the correct degree of compaction of the soil around the porous cup. To attain this with maximum accuracy, it is advisable to obtain a sharp metal cylinder very slightly smaller in diameter than the vertical-walled porous cup and drive this into the soil between the seedling rows, remove the core, and then place the porous cup into the hole with a twisting motion. Soil can then be packed on top of the pot to level it off with the surface.

The field method of calibration outlined above has been found reasonably satisfactory but it has a disadvantage in that a calibration curve, for at least 90 per cent. of the range of moisture content in which plants will survive, may not be obtained for many months.

An alternate method of calibration is to obtain a jar of soil (from 0.5 to 1 gallon in volume) from the nursery area at which the tensiometer is to be set up and to do the calibrating in the laboratory. In the laboratory method of calibration the soil is thoroughly saturated, then packed around the porous cup. The top of the tin container is covered with a double layer of tight-fitting building paper, and the reading obtained next day, or as soon as it is evident that the moisture is evenly distributed and an equilibrium has been established. The cover is then removed and the entire tin with enclosed soil mass and porous cup is weighed. The soil is then allowed to dry out gradually, with readings being taken and weighings made morning and night until the soil appears quite dry and maximum mercury lifts of 30 to 60 cm. (depending on soil texture) have been recorded. The porous cup is then removed and the entire soil mass oven-dried at 105° C. The net oven dry weight of the soil is obtained and total moisture-content values for the entire range of the calibration are computed. This proved the most satisfactory method of calibration and results for any one soil could be obtained in a 2- to 4-week period.

A second system of calibration in the laboratory was tried in which rather dry soil was packed around the porous cup in a large tin can, and the corresponding capillary tension recorded. Thirty to forty ml. of water were then added to the soil mass and a small portion of the soil was placed in a tight can for oven-drying. The process was repeated until a series of readings over a range of moisture contents had been obtained.

This method was discontinued because of the fact that changing volume-weight of the soil caused by repeated removal and repacking of the soil gave a rather wide dispersion of points for the average calibration curve. Moreover, since calibration is done on a rising moisture curve, the error incurred due to the hysteresis effect was correspondingly greater when the tensiometers were set up in the field, because, as pointed out by HAINES (**4**), the conditions in the field are generally represented by a falling moisture curve. This error for sandy soils usually amounts to 1 to 2 per cent. of moisture as read on the scale; for fine-textured soils it is considerably higher.

Experimental results

Figure 2 shows the calibration points and fitted curves obtained for six different soils. The physical properties of these soils are given in table I, where they are arranged in ascending order of texture. It will be noted that these appear in exactly the same order from left to right on figure 2. Of especial interest is the fact that coarse-textured material like soil no. 1, which is classified as a medium sand, exerts very little suction pull until it is within 3 or 4 per cent. of the computed wilting coefficient, and then increases very rapidly as it approaches the wilting coefficient. For the loamy sands and sandy loams the rise is somewhat more gradual, and for a silt loam it is even more uniform.

The maximum pull recorded in the calibration of the tensiometers was 54 centimeters. Beyond that point, air entered through the side walls of the porous pot and collected in sufficient volume in the system to make the readings unreliable. For forest nurseries located on sandy soils, however, a working range of 0 to 50 cm. of mercury pull is entirely adequate since it covers practically the entire range of moisture conditions encountered in nurseries in the periods of spring germination, active early summer growth, and hardening-off in late summer.

EFFECT OF ORGANIC MATTER ON CAPILLARY TENSION

In connection with the calibration of tensiometers, the rather practical question has come up as to whether or not such an instrument, once calibrated for a given spot in the nursery, could not be used year after year in that same location. Data on performance of such moisture meters over a period of two years indicate that this can be done safely, provided no heavy

TABLE I

PHYSICAL PROPERTIES OF SOILS USED IN MOISTURE-METER TESTS

Soil and curve number	Nursery	Instrument number	Mechanical analysis									Moisture constants		
			Fine gravel 2.0–1.0 mm.	Coarse sand 1.0–0.50 mm.	Medium sand 0.50–0.25 mm.	Fine sand 0.25–0.10 mm.	Very fine sand 0.10–0.05 mm.	Total silt + clay. Below 0.05 mm.	Fine material separates			Moisture equivalent*	Wilting coefficient†	Hygroscopic coefficient‡
									Silt 0.05 to 0.005 mm.	Clay 0.005 to 0.002 mm.	Fine clay. Under 0.002 mm.			
			%	%	%	%	%	%	%	%	%	%	%	%
1	Hayward, Wis.	39	4.1	21.7	37.1	22.8	4.9	9.4	5.0	0.8	3.6	4.0	2.2	1.5
2	Wellston, Mich.	38	0.8	8.1	40.1	35.0	3.8	12.2	6.8	1.4	4.0	5.7	3.1	2.1
3	Wellston, Mich.	37	1.0	8.9	39.2	35.2	2.4	13.3	6.9	1.3	5.1	7.8	4.2	2.9
4	Butternut, Wis.	41	0.8	2.0	17.7	50.0	14.2	15.3	9.3	0.9	5.1	8.5	4.6	3.1
5	Knife River, Minn.	35	10.8	15.9	18.0	21.3	7.2	26.8	14.9	1.3	10.6	14.2	7.7	5.2
6	Licking, Mo.	36	1.1	2.5	9.8	17.9	3.1	65.6	42.4	8.4	14.8	19.1	10.4	7.0

* Represents percentage of moisture held against a force equal to 1,000 times force of gravity, and determined by standard centrifuging method. Determined by Division of Soils, University of Minnesota.

† Computed from formula: Wilting coefficient $= \frac{\text{moisture equivalent}}{1.84}$. This value represents point at or near which temporary wilting occurs on most conifers growing under nursery conditions. Plants will recover if watered shortly after wilting occurs.

‡ Computed from formula: Hygroscopic coefficient $= \frac{\text{moisture equivalent}}{2.71}$. This value represents point at which all trees die if exposed for any appreciable length of time. Serious mortality often occurs, even before moisture drops to the hygroscopic coefficient.

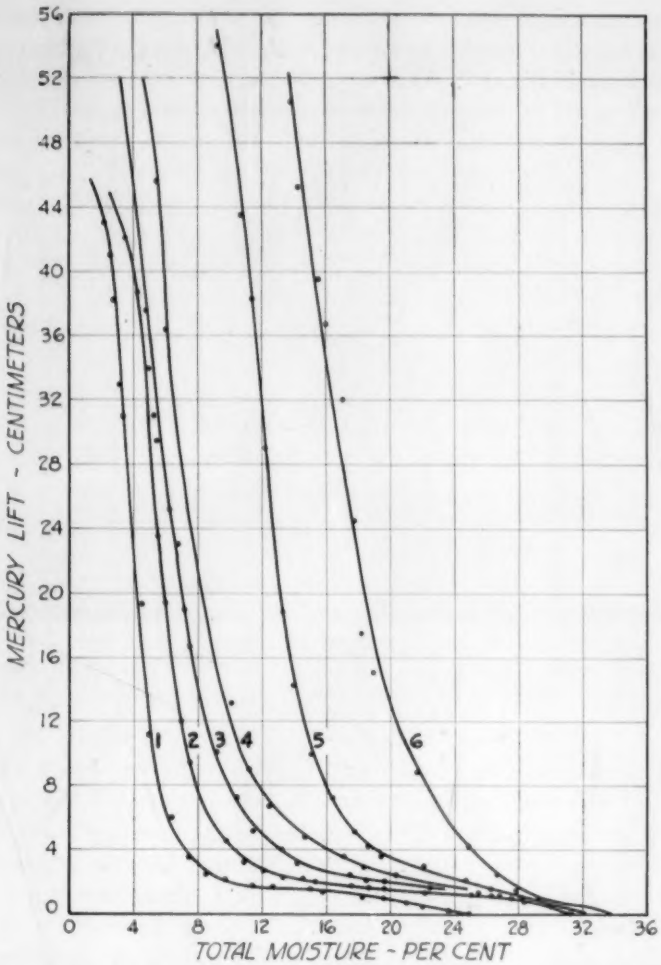

FIG. 2. Calibration curves obtained when tensiometers calibrated in soils ranging from sand (left) to silt loam (right). A Red Wing pot was used as the porous element.

addition of organic matter is made such as is provided by peat, compost, animal manures, and possibly soiling crops. Some error, however, will occur in the accuracy of the instruments because the settling effect of irrigation and rain gradually causes a compaction of the seedbed, which at the time of sowing is in a loosely packed condition. This change in volume-weight as the season progresses necessarily causes a slight error in readings of the total moisture percentage.

The soil-maintenance program in the Federal and State forest nurseries in the Lake States requires the addition of rather large quantities of peat and

compost after removal of a crop of trees. The addition of such material will cause a marked difference in the type of calibration curve obtained for sandy soils, as is shown in figure 3.

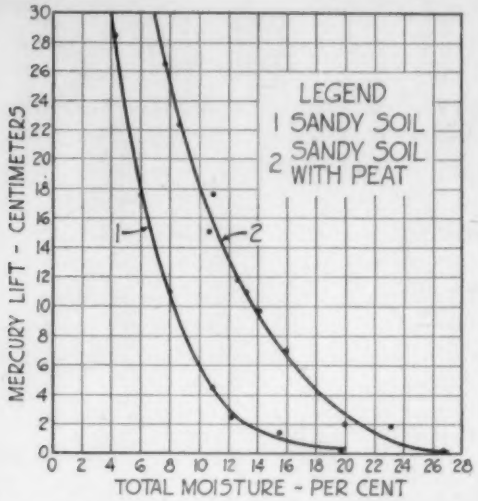
LEGEND
1 SANDY SOIL
2 SANDY SOIL WITH PEAT
MERCURY LIFT - CENTIMETERS
TOTAL MOISTURE - PER CENT
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30

FIG. 3. Effect of peat on calibration curve of a sandy nursery soil containing 12 per cent. of silt plus clay. The soil for curve no. 2 was the same as used for curve no. 1, except that it contained 1 pound of peat (oven-dry basis) for every 20 pounds of soil, a treatment equivalent to about 50 tons per acre of dry peat plowed into the top 6 inches of soil.

In this case the addition of 50 tons per acre of acid peat (oven dry-weight basis) obtained from a spruce-tamarack swamp changed the calibration curve from that of a rather typical fine sand to the type of curve ordinarily obtained for a light sandy loam. The explanation for this is quite simple. The addition of a large quantity of organic matter increases the number of contact points and the pore space. As a consequence, in a soil of equal total moisture content, it decreases the radius of curvature of the wedges of water that occur between the particles of soil. This results in an increase of the capillary tension of the soil if held at the same total moisture per cent.

Discussion

Interpretation of calibration curves

From the data obtained from the calibration curve it is an easy matter to make a scale for the tensiometer showing total moisture content and the wilting coefficient. These scales are attached to the tensiometers in place of the centimeter scale, and thereafter the nurseryman can readily compute the moisture content of that portion of the nursery by reading the total

moisture content and subtracting from it the indicated wilting coefficient to obtain the percentage of available[7] moisture in the soil.

As an aid to nurserymen in judging relative soil-moisture in its relation to active growth, table II incorporates tensiometer information for the better sands and loamy sand nursery soils which are the usual textures in most Federal and State nurseries found in the Lake States. The data are based on observations with tensiometers at Rhinelander and Hayward, Wisconsin, and Manistique, Michigan. Similar tables can be made up for the sandier as well as the finer-textured soils, but these would require some adjustment in the second and third columns, especially for the fine-textured soils. Refinements are necessary for individual species and are yet to be worked out.

It will be noted that when the mercury depression exceeds 35 centimeters, the moisture content for the loamy sand soils is at, or within, a few per cent. of the wilting coefficient; watering will thus be necessary soon after this pull is attained. When the tension is between 25 and 35 cm., growth (especially of tops) is slow, and between 15 and 25 it is fair to good. Growth is best when the suction pull is between 2 and 15 cm. When the capillary tension is less than about 2 cm., the soil is definitely soggy and sometimes even waterlogged. Fortunately, in the sandy soils of most Lake States nurseries an excess of water due to heavy rains is soon lost by underdrainage, and a saturated condition does not occur for more than a few hours.

The correlation of the type of calibration curve obtained with the texture and moisture equivalent of soils suggests the possibility of dispensing with the calibration of the moisture meters. On the basis of a mechanical analysis of the soil and a determination of the moisture equivalent, it might be feasible to make up a scale showing relative soil-moisture status of the soil, using the terms similar to those given in column 1 of table II. This would, however, require access to a chart showing calibration curves of at least 30 to 50 soils having a range of silt-plus-clay content from 2 per cent. to 90 per cent. and would, furthermore, be contingent on complete information on effect of packing, structure, organic matter, soil nutrients, and volume weight of the soil and their effect on tensiometer readings under field conditions. For the present, the safest procedure will necessarily rely on a calibration of each instrument for every field setup since the individual and composite effect of the above factors has not been determined to any degree of refinement.

[7] "Available" water as expressed here refers to the percentage of moisture above the computed wilting coefficient. In reality some of the water between the computed hygroscopic and wilting coefficient values can be extracted by conifers but this moisture, is of no consequence in producing any appreciable growth, as it is barely adequate to sustain life for a comparatively short time in the mesophytic type of plants. This is especially true for the species of pine and spruce commonly grown in nurseries in Michigan, Wisconsin and Minnesota.

TABLE II

CORRELATION OF TENSIOMETER READINGS WITH MOISTURE CONTENT AND GROWING CONDITIONS IN LOAMY SAND SOIL USED FOR PRODUCTION OF CONIFERS

SOIL MOISTURE STATUS	MERCURY PULL	APPROXIMATE AVAILABLE MOISTURE CONTENT	REMARKS
	cm.	%	
Very dry	Over 35	0–2	Conifers are at or near wilting coefficient. Serious mortality may occur if water is not applied. Moisture should not be held in this range for prolonged periods during active growth period
Dry	25–35	2–4	Use this range principally in hardening stock
Fresh	15–25	4–6	Allow moisture to reach this range, or slightly lower, even during active growing season, to induce drought hardiness
Moist	2–15	6–14	Active top and root growth occurs
Wet	Under 2	Over 14	Too moist. Do not maintain moisture in this range for any protracted length of time in conifer nurseries

Experience, backed by experimental evidence, indicates that in nursery practice the moisture content of the top eight inches of soil should be maintained mostly in the "fresh" and in the drier part of the "moist" range. This 8-inch level is specified because in most Lake States nurseries usually from 70 to 95 per cent. of the root system of seedling and transplant conifers is found in the upper eight inches. Consequently, in using tensiometers in conifer nurseries, the porous cups are usually set so that the absorbing surface is somewhere in the zone indicated. For shallow-rooted or first-year seedlings, the cup is generally placed at the 1- to 4-inch zone, while for stock 2 years of age or older, the cup is set at the 2- to 5-inch or 3- to 6-inch zone.

In the latter part of the growing season, which in the Lake States means August 15 to September 15, the moisture content should be held, if conditions permit, in the "dry" and "fresh" range, in order to harden the stock properly. The hardening process decreases moisture content of the stock, reduces rate of top growth and leaf surface, and accelerates the root development of the plant. Thus a comparatively low top-root ratio results and the well-balanced plants so produced have a better chance of surviving drought and winter killing. A comparatively low soil moisture content in conifer beds in late summer will also hasten dormancy and increase frost resistance.

Performance of tensiometers in forest nurseries

Figures 4 and 5 give the records of performance of two tensiometers in forest nurseries, one at Rhinelander, Wisconsin, and the other at Manistique,

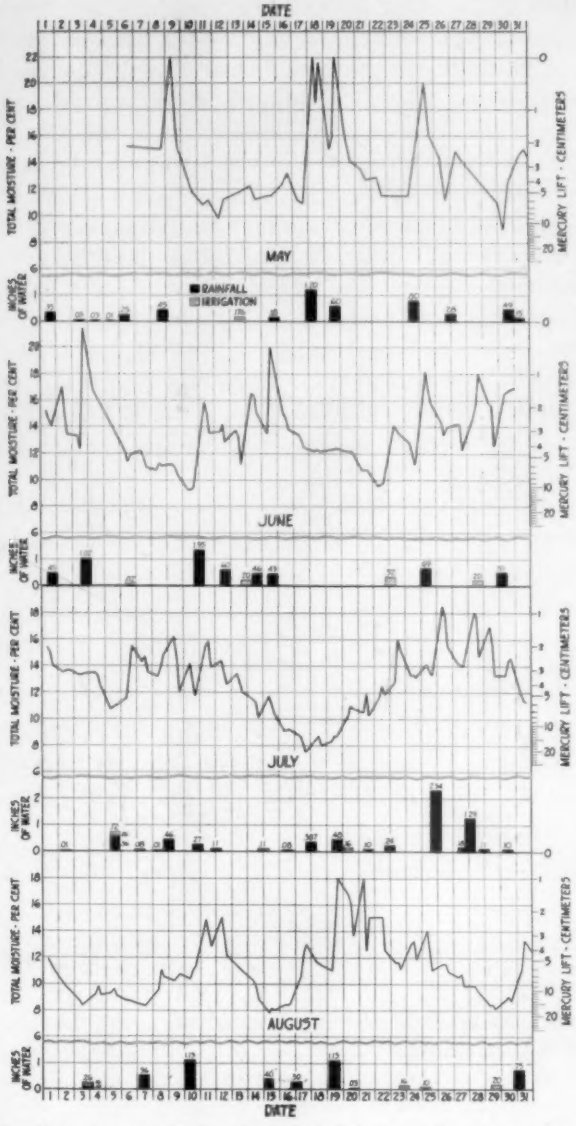

Fig. 4. Soil moisture content at a depth of 4 inches of a loamy fine sand soil as determined by a tensiometer in an experimental bed of 2–0 jack pine, Hugo Sauer Nursery, Rhinelander, Wisconsin, 1938. The soil had a silt plus clay content of 15 per cent., a moisture equivalent of 8 per cent., and a computed wilting coefficient of 4.3 per cent.

Michigan.[8] Readings were generally taken at least once, and often twice daily. The soils in these nurseries are a loamy fine sand and a fine sand respectively.

The correlation between tensiometer readings and the frequency and amount of precipitation and irrigation water received is noteworthy. It will also be noted that each of the soils had a fairly definite "tapering off" zone in the moisture curve, which is about 14 per cent. and 7 per cent., respectively, for Rhinelander and Manistique nurseries. This value for both soils lies between 1.50 and 1.75 times the moisture equivalent value. In other words, each soil has a certain field holding capacity and any water applied in excess of this amount drains away very rapidly into the subsoil. This rapid drop of soil moisture content after the soil has received a thorough soaking by precipitation or irrigation is strikingly illustrated in figure 5 and is brought out in more detail in figure 6, which shows a drop from 23 to 15 per cent. in total moisture content in the brief period of 6 hours, largely because of loss by underdrainage. There was apparently very little water loss by evaporation or transpiration from 5 P.M. of one day to 8 A.M. of the next, but after 9 A.M. on the second day these forces again became active in causing water loss.

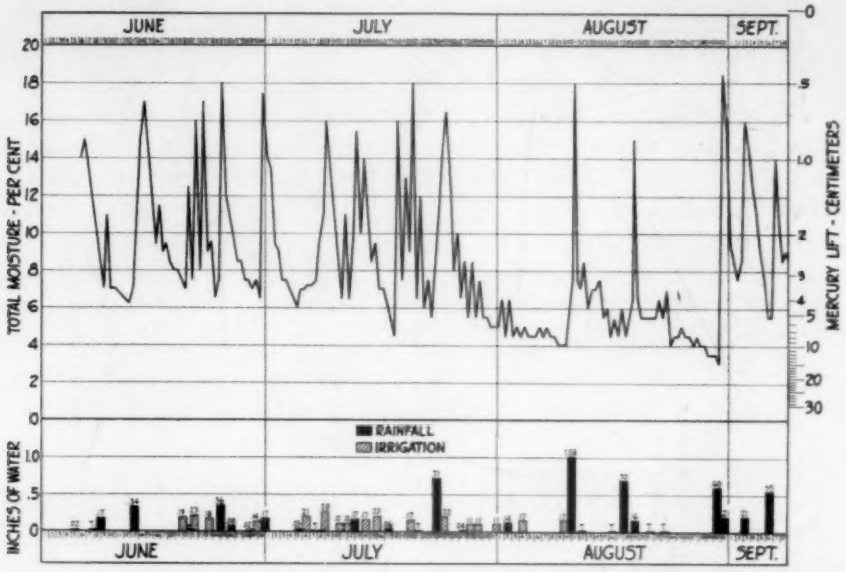

FIG. 5. Soil moisture content at a depth of 3 inches of a fine sand soil as determined by a tensiometer in a bed of 2–0 red pine, Wyman Nursery, Manistique, Michigan, 1938. The soil had a silt-plus-clay content of 1.8 per cent., a moisture equivalent of 3.8 per cent., and a wilting coefficient of 2.1 per cent.

[8] The record for Manistique was furnished through the cooperation of Messrs. E. F. BIEBESHEIMER and FLOYD STRUBLE, Nursery Superintendent and Assistant Superintendent respectively.

Value of Tensiometers

The tensiometers have practical value to the nurseryman. He can control water supply and thus provide drought-hardy planting stock and prevent wastage of water by unnecessary irrigation and subsequent loss of highly soluble fertilizers, such as ammonium sulphate and potash. Even though the soluble fertilizers are composted and partially adsorbed with peat, muck, or other organic materials, some loss is certain to occur and it will be accelerated by excessive watering.

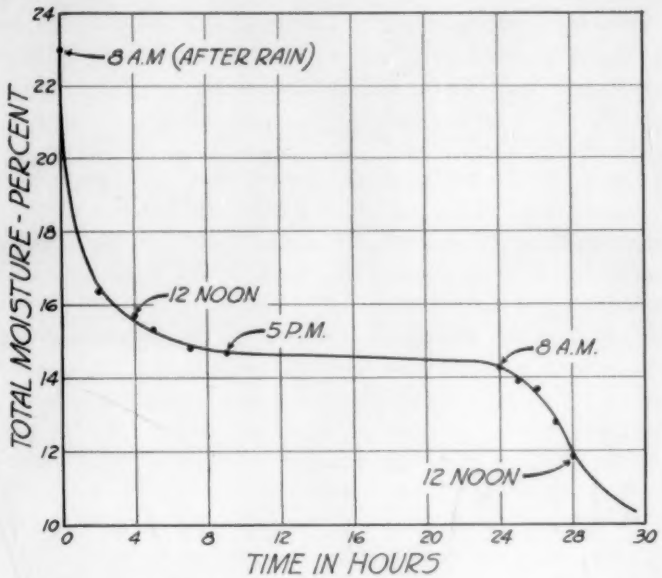

Fig. 6. Graph illustrating loss of moisture in a loamy fine sand soil, Hugo Sauer Nursery, Rhinelander, Wisconsin, August 3 and 4, 1937. Porous cup of tensiometer was 2 inches below surface of bed of 1–0 jack pine.

The tensiometers are equally valuable in periods of severe drought; they indicate depth of penetration of irrigation water, especially if the porous cups are set at several depths in the rooting zone of the seed bed. Thus they indicate the effectiveness of the water applied and furnish warning of impending injury or actual death of nursery stock because of low soil moisture content. Such a drought occurred in 1936 in the Lake States. High temperatures combined with markedly subnormal rainfall resulted in rather sudden losses in nurseries, especially in the center of the second-year seedbeds of pine and spruce. A very low soil moisture content is not, of course, conducive to the best growth of conifers. It is necessary to strike a happy medium between the two extremes mentioned.

It is obvious from the records obtained in this study that tensiometers

can be of valuable assistance in understanding fully the rôle of moisture in forest nurseries or in experimental plots where the moisture content of the soil is being studied in relation to drought hardiness, growth, and yield. To obtain a similar detailed record with the usual method of soil sampling and oven drying would require a large amount of work. Moreover, in some experimental work it would be very undesirable to disturb the plots by removing several hundred soil samples over a period of three or four months.

Instruments based on the tensiometer principle will, no doubt, be of increasing value in the field of plant physiology, floriculture, horticulture, agronomy, ecology, and hydrology. There is a definite need for commercial development of such an instrument, which would combine the best features of the tensiometers built by various investigators.[9]

Summary

A description is given of several types of tensiometers and of their use in gauging the necessity of watering in conifer nurseries in the Lake States. Records are presented showing the performance of two of the instruments for a period of three to four months in two different nurseries. Calibration technique is described and the capillary potential of soil is shown to vary by texture and by addition of organic matter. A discussion is given to show how watering may influence development of nursery stock, especially as regards size, top-root ratio, frost hardiness, and drought resistance.

Practical values of this type of "moisture meter" to the nurseryman include: (1) prevention of injury or death of trees due to the warning given of the presence of low soil moisture content in periods of heat and droughts; (2) aid in the regulation of moisture, especially during the "hardening-off" period, in such a manner as to obtain planting stock that is frost resistant and drought hardy; (3) the prevention of excessive watering with concomitant expenses and the subsequent loss, by underdrainage, of highly soluble commercial fertilizers.

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LITERATURE CITED

1. BOUYOUCOS, G. J. Evaporating the water with burning alcohol as a rapid means of determining moisture content of soils. Soil Sci. **44**: 377–383. 1937.
2. HEATH, O. V. S. A method of water control for sand cultures. Ann. Bot. **43**: 71–79. 1929.
3. HECK, A. F. A soil hygrometer for irrigated cane lands of Hawaii. Jour. Amer. Soc. Agron. **25**: 272–278. 1934.

[9] Instruments of this type are already being manufactured commercially in England.

4. HAINES, W. B. Studies in the physical properties of soil: V. The hysteresis effect in capillary properties and the modes of moisture distribution associated therewith. Jour. Agr. Sci. **20**: 97–116. 1930.
5. KORNEV, V. G. The suction force of soils. Zhurnal Opitnoi Agron. (Russian Jour. Exp. Agron.) **22**: 105–111. (1921–23). 1924. Abstract in Soil Sci. **17**: 428. 1924.
6. LIVINGSTON, BURTON E., and NOREM, W. LUTHER. The water-supplying power and water-absorbing power of soil as related to wilting of wheat and Coleus in greenhouse pot cultures. Soil Sci. **43**: 177–204. 1937.
7. LYNDE, C. J., and DUPRÉ, H. A. On a new method of measuring the capillary lift of soils. Jour. Amer. Soc. Agron. **5**: 107–116. 1913.
8. RICHARDS, L. A. The usefulness of capillary potential to soil moisture and plant investigators. Jour. Agr. Res. **37**: 719–742. 1928.
9. ————————, and GARDNER, WILLARD. Tensiometers for measuring the capillary tension of soil water. Jour. Amer. Soc. Agron. **28**: 352–358. 1936.
10. ————————, and NEAL O. R. Some field observations with tensiometers. Proc. Soil Sci. Soc. Amer. **1**: 71–91. 1936.
11. ROGERS, W. S. A soil moisture meter depending on the capillary pull of the soil. Jour. Agr. Sci. **25**: 326–343. 1933.
12. SHIRLEY, H. L. A method of studying drought resistance in plants. Science n.s. **79**: 14–16. 1934.
13. SMITH, W. O. Sorption in an ideal soil. Soil Sci. **41**: 209–230. 1936.
14. STOECKELER, J. H. Measuring soil moisture content in the forest nursery. Preliminary statement issued for the use of field men. Lake States Forest Experiment Station. Mimeographed. July 30, 1937.

THE EFFECT OF VARIOUS WAVEBANDS OF SUPPLEMENTARY RADIATION ON THE PHOTOPERIODIC RESPONSE OF CERTAIN PLANTS[1]

R. B. WITHROW AND ALICE P. WITHROW

(WITH SIX FIGURES)

Introduction

The effect of various wavebands of visible radiation, used to lengthen short daylight periods, on the photoperiodic response of plants has been the subject of considerable disagreement. SCHAPPELLE (**9**) concludes that supplemental red and blue radiation are equally effective in inducing flowering in radish and spinach, that red is more effective than blue in the case of China aster and early flowering cosmos and that blue is more effective than red in the case of lettuce, although red induces flowering. Salvia, chrysanthemum, Kalanchoe, teosinte, and Maryland Broadleaf tobacco failed to flower under any radiation prolonging the daylength to more than ten hours.

FUNKE (**3, 4, 5**) divided his results into four types of flowering response when he supplemented an 8-hour day with red, blue, and white radiations. Group I included those plants which flowered under short days and blue at the same time and under white and red at the same time; plants of group II failed to flower under short days but flowered under all long day conditions; those in group III flowered only under white radiation and under no other condition; and those in group IV flowered simultaneously under white and blue radiation and under red and dark at the same time. He used a light intensity of from 30 to 60 lux (approximately 3 to 6 foot candles) and a temperature of 12° to 15° C.

RASUMOV (**8**) found that, in general, red light acted like white light in inducing flowering in long day plants and inhibiting flowering in short day plants; green, blue, and violet light acted like darkness. He found, however, a variable sensitivity to differences in wavelengths of radiation.

WITHROW and BENEDICT (**11**), and WITHROW and BIEBEL (**12**) found that red and white radiation induced early flowering in long day plants and inhibited flowering in short day plants. Short day plants flowered in days lengthened with green and blue light, as well as under short days. Some long day plants, such as China aster and *Helianthus cucumerifolius* also flowered under blue radiation at the same time as under the red, while Scabiosa, another long day plant, failed to flower under the blue. The plants were grown at 50° F. night and 55° F. day temperature.

[1] Contribution from the Department of Horticulture, Indiana Agricultural Experiment Station, Lafayette, Indiana.

These results appear to disagree chiefly on the effect of the blue radiation. Most of the workers agree that red radiation is effective in inhibiting flowering in short day plants and inducing flowering in long day plants.

Considering that the flowering responses of many long and short day plants are strongly influenced by very low intensities of incandescent lamp radiation (**1, 11**) applied to prolong short winter days, it is to be expected that the more sensitive plants would be affected by very small amounts of red light transmitted by the blue filters and by unfiltered radiation scattered from the radiation equipment. It has been shown (**11**) that only a few hundredths of a foot candle are sufficient to induce the flowering of China aster and intensities above 0.5 foot candle inhibits flowering in Biloxi soybean (**1**). Unless very special precautions are taken in the design of radiation equipment, extraneous red radiation may mask the effect of the blue band transmitted by the filter system and the result might apparently indicate a second maximum in the spectral response curve of the most sensitive types of plants. Less sensitive plants would probably not be appreciably influenced by these extraneous radiations and a single maximum in the red would occur.

The present investigation, therefore, is an attempt to determine the relative effect of supplemental blue radiation on the flowering response of certain long and short day plants and to further corroborate results secured with the longer wavelengths of visible radiation.

Procedure

The plants were grown in a greenhouse in an automatic sub-irrigation gravel culture system (**13**) with the nutrient solution shown in table I. The

TABLE I

NUTRIENT SOLUTION FORMULA

SALT	CONCENTRATION-MILLI-MOLAR
$MgSO_4$	1
$Ca(H_2PO_4)_2$	1
KNO_3	10
K_2SO_4	5
$CaSO_4$	8

solution was pumped into the beds on 4-hour cycles. The pH was maintained between 5.5 and 6.5. The temperature was manually controlled at approximately 62–65° F. day and 52–55° F. night.

Callistephus chinensis, variety Heart of France (China aster), *Scabiosa atropurpurea*, variety Azure Fairy, *Spinacia oleracea*, variety Nobel (spinach), *Soja max*, variety Mukden (soybean), *Xanthium pennsylvanicum*, *Salvia splendens*, variety Fairly Tall, and *Tithonia speciosa* were the plant materials used in the experiment. All seed except soybean were sown

in sand flats. The sand flats of long day plants were placed immediately after seeding under short day conditions; those of short day plants were placed under a 24-hour day of daylight supplemented with 20 foot candles of radiation from an incandescent tungsten filament lamp in order to prevent induction of floral buds. The seedlings were transplanted into the radiation plots in the gravel benches as soon as they were large enough. Soybean was sown directly into the plots of gravel.

Five radiation conditions were included in the investigation. The short day plot received daylengths as given in table II. The other four plots received red, yellow-green, and blue radiation of approximately 100 ergs/cm.^{2}/second, and blue of 400 ergs/cm.^{2}/second to supplement the natural daylength to a 24-hour day. No dark period was given the plants in the radiation plots. The lamp and filter equipment, together with transmission data, are presented in table III and figure 1. For the blue, the two most intense blue mercury lines at 4047Å and 4358Å were used and for the yellow-green, the green line at 5461Å, and the yellow doublet at 5770Å and 5791Å. The radiation was measured by means of a thermopile calibrated against a standard lamp of the U. S. Bureau of Standards.

TABLE II

DAYLENGTH FROM OCTOBER 1 TO MAY 1, LAYFAYETTE, INDIANA

DATE	DAYLENGTH	
	hr.	*min.*
Oct. 1	11	46
Oct. 15	11	10
Nov. 1	10	28
Nov. 15	9	57
Dec. 1	9	30
Dec. 15	9	18
Dec. 21	9	16
Jan. 1	9	20
Jan. 15	9	36
Feb. 1	10	9
Feb. 15	10	41
Mar. 1	11	17
Mar. 15	11	54
Apr. 1	12	40
Apr. 15	13	16
May 1	13	56

The gelatin filters consisted of dyed gelatin poured onto panes of 20-inch square window glass and placed in a light-tight rack soldered to the bottom of the primary filter cells. Each primary filter cell consisted of a sheet-iron tank 6 inches deep and 22 inches square, having an open bottom into which a pane of glass was sealed with asphalt. The interior of the tank was painted with asphalt to protect it from the corrosive action of acidulated copper sul-

TABLE III

DATA ON RADIATION EQUIPMENT

BAND AND INTENSITY	WAVELENGTH LIMITS	LIGHT SOURCE	PRIMARY FILTER; AQUEOUS	SECONDARY FILTER OF DYED GELATIN
ergs/cm.²/sec.	*A*			
Blue 100	4047 and 4358 Hg lines	250-watt type H_2, high pressure Hg arc	10 cm. 2 per cent. $CuSO_4 \cdot 5H_2O$	Victoria pure blue BO and crystal violet
Blue 4X 400	4047 and 4358 Hg lines	400-watt type H_1, high pressure Hg arc	10 cm. 2 per cent. $CuSO_4 \cdot 5H_2O$	Victoria pure blue BO and crystal violet
Yellow-green 100	5461, 5770 and 5791 Hg lines	400-watt type H_1, high pressure Hg arc	10 cm. 4 per cent. $CuSO_4 \cdot 5H_2O$	Pontamine fast yellow 5GL and orange G
Red 100	6400 to approximately 9000. Continuous spectrum	500-watt gas filled tungsten filament lamp	10 cm. 1 per cent. $CuSO_4 \cdot 5H_2O$	Chrysoidine Y and crystal violet

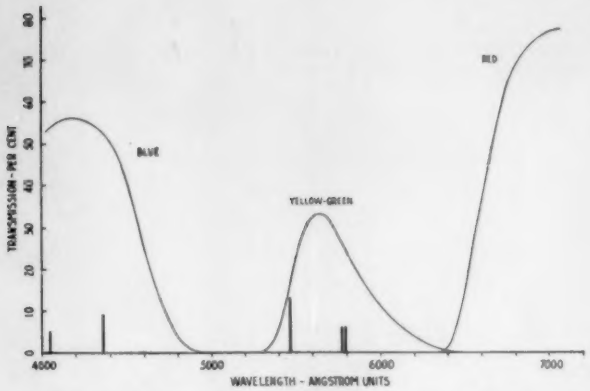

FIG. 1. Percentage transmission of filter systems.

phate solutions. The top of the filter cell was covered with a lid having a circular opening 17 inches in diameter upon which rested the 18-inch diameter porcelain enamelled low bay type of reflector containing the appropriate lamp. It was possible to eliminate practically all extraneous light with this equipment.

The individual experimental plots were separated from each other with opaque black rubberized cloth. The leaf areas were measured with a photoelectric surface type photometer having a 12-inch aperture (**10**). The plant material was harvested, weighed and dried at 100° C. in a forced draft thermostatically controlled drying oven for about 14 hours.

Results

Data are presented on the date of appearance of macroscopic flower buds, date of flower opening, on height and weight and, in the case of aster and soybean, on leaf number and area. These data are given in tables IV through X. Photographs of the plant material are shown in figures 2, 3, 4, 5, and 6. The height, weight and leaf area data were taken from 50 to 70 days after seeding, depending on the germination and growth rate of the plant. Flowering data on aster were taken into the spring months when the natural daylength conditions were long enough to induce flowering in aster without the addition of artificial light.

All the long day plants set bud under red and yellow-green light. Scabiosa and spinach failed to set bud under blue light or the short day condition. The short day and blue plots of aster were continued into the spring months when the daylength was around 12 hours. Under these daylengths, aster showed a slight decrease in budding time under blue radiation, a greater decrease occurring under the higher intensity blue, as compared to those plants grown in natural daylengths.

TABLE IV

FLOWERING AND WEIGHT DATA ON *Callistephus chinensis*, VARIETY HEART OF FRANCE
SEEDING DATE: OCTOBER 27, 1938
IRRADIATION DATES: DECEMBER 12, 1938, TO HARVEST
HARVEST DATE: JANUARY 13, 1939
NUMBER OF PLANTS PER TREATMENT: 40 FOR WEIGHT, AND 20 FOR FLOWERING DATA

TREATMENT	No. DAYS TO BUD*	No. DAYS TO FLOWER*	AV. BUDS AND FLOWERS	AV. HEIGHT	AV. DRY WEIGHT			DRY MATTER	TOP/ ROOT RATIO	AV. NO. LEAVES	AV. LEAF AREA/ LEAF	AV. LEAF AREA/ PLANT
					TOP	ROOT	TOTAL					
				cm.	*mg.*	*mg.*	*mg.*				*cm.*^{2}	*cm.*^{2}
Short day	102	140	47	0.3	170	63	233	8.4	2.7	16	3.7	60
Blue	94	135	38	0.3	222	70	292	8.9	3.1	16	4.6	74
Blue 4X	90	123	26	0.5	203	51	254	8.2	4.0	12	6.0	75
Yellow-green	58	107	10	0.5	221	48	269	7.7	4.6	11	7.6	84
Red	58	101	8	4.9	266	54	320	7.8	4.9	11	9.4	103

* From beginning of long day treatment.

TABLE V

FLOWERING AND WEIGHT DATA ON *Scabiosa atropurpurea*, VARIETY AZURE FAIRY

SEEDING DATE: OCTOBER 7, 1938
IRRADIATION DATES: DECEMBER 2, 1938, TO HARVEST
HARVEST DATE: JANUARY 1, 1939
NUMBER OF PLANTS PER TREATMENT: 40 FOR WEIGHT AND 20 FOR FLOWERING DATA

TREATMENT	No. DAYS TO BUD*	No. DAYS TO FLOWER*	AV. HEIGHT	AV. DRY WEIGHT			DRY MATTER	TOP/ ROOT RATIO
				TOP	ROOT	TOTAL		
			cm.	*mg.*	*mg.*	*mg.*	%	
Short day	.....	.....	2.9	471	111	582	7.4	4.2
Blue	.....	.....	3.4	555	145	700	7.3	3.8
Blue 4X	.....	.....	2.9	475	116	591	7.0	4.1
Yellow-green	110	.....	4.0	478	108	586	7.1	4.4
Red	61	99	14.5	545	101	646	7.5	5.4

* From beginning of long day treatment.

The short day plants all failed to form macroscopic flower buds under red and yellow-green radiation and all set bud under both intensities of blue light and under the short day treatment. The low intensity blue affected the budding time either not at all or increased it slightly. The higher intensity delayed the budding time from 20 to 50 per cent. in all the short day plants with the exception of Salvia.

With aster, Scabiosa, Xanthium and soybean, all the radiation treatments increased the total dry weight over that of plants in the short day. Spinach

TABLE VI

FLOWERING AND WEIGHT DATA ON *Spinacia oleracea*, VARIETY NOBEL

SEEDING DATE: OCTOBER 27, 1938
IRRADIATION DATES: DECEMBER 13, 1938, TO HARVEST
HARVEST DATE: JANUARY 20, 1939
NUMBER OF PLANTS PER TREATMENT: 20

TREATMENT	No. DAYS TO BUD*	No. DAYS TO FLOWER*	AV. HEIGHT	AV. DRY WEIGHT			DRY MATTER	TOP/ ROOT RATIO
				TOP	ROOT	TOTAL		
			cm.	*mg.*	*mg.*	*mg.*	%	
Short day	.....	.....	0.4	505	77	582	9.3	6.4
Blue	.....	.....	0.5	478	66	544	9.6	7.2
Blue 4X	.....	.....	0.6	457	63	520	8.3	7.3
Yellow-green	20	34	27.4	872	113	985	7.8	7.8
Red	20	34	35.8	870	104	974	8.2	8.4

* From beginning of long day treatment.

TABLE VII

FLOWERING AND WEIGHT DATA ON *Soja max*, VARIETY MUKDEN
SEEDING DATE: DECEMBER 10, 1938
IRRADIATION DATES: GERMINATION TO HARVEST
HARVEST DATE: FEBRUARY 3, 1939
NUMBER OF PLANTS PER TREATMENT: 20

TREATMENT	No. DAYS TO BUD	No. DAYS TO FLOWER	AV. HEIGHT	AV. DRY WEIGHT			DRY MATTER	TOP/ ROOT RATIO	AV. NO. LEAVES	AV. LEAF AREA/ PLANT	AV. LEAF AREA/ LEAF
				TOP	ROOT	TOTAL					
			cm.	*mg.*	*mg.*	*mg.*	%			*cm.*²	*cm.*²
Short day	41	85	13.3	546	56	602	15.1	9.7	5.6	16.1	90
Blue	43	87	19.5	676	63	739	16.1	10.7	6.1	19.7	120
Blue 4X	62	100	21.9	564	52	616	13.7	10.8	5.2	21.6	112
Yellow-green	.....	.....	43.6	805	75	880	11.7	10.7	6.2	22.5	140
Red	.....	.....	45.2	600	45	645	11.8	13.4	5.7	23.2	131

TABLE VIII

FLOWERING AND WEIGHT DATA ON *Xanthium pennsylvanicum*
SEEDING DATE: JANUARY 5, 1939
IRRADIATION DATES: GERMINATION TO HARVEST
HARVEST DATE: MARCH 17, 1939
NUMBER OF PLANTS PER TREATMENT: 10

TREATMENT	No. DAYS TO BUD	No. DAYS TO FLOWER	AV. HEIGHT	AV. DRY WEIGHT			DRY MATTER	TOP/ ROOT RATIO
				TOP	ROOT	TOTAL		
			cm.	*mg.*	*mg.*	*mg.*	%	
Short day	33	57	24.8	3720	590	4310	12.2	6.3
Blue	33	57	32.0	3680	610	4290	13.2	6.0
Blue 4X	47	71	35.5	3100	610	3710	11.1	5.1
Yellow-green	.....	.....	40.3	3670	690	4360	11.1	5.3
Red	.....	.....	43.3	3690	720	4410	11.5	5.1

TABLE IX

FLOWERING AND WEIGHT DATA ON *Xanthium pennsylvanicum*
SEEDING DATE: JUNE 26, 1939
IRRADIATION DATES: GERMINATION TO HARVEST
HARVEST DATE: JULY 15, 1939
NUMBER OF PLANTS PER TREATMENT: 30

TREATMENT	No. DAYS TO BUD	AV. HEIGHT	AV. DRY WEIGHT			DRY MATTER	TOP/ ROOT RATIO
			TOP	ROOT	TOTAL		
		cm.	*mg.*	*mg.*	*mg.*	%	
9 hour day	14	7.3	355	46	401	9.0	7.7
24 hour day	.....	9.6	289	41	330	9.0	7.2

TABLE X

FLOWERING DATA ON *Salvia splendens*, VARIETY FAIRLY TALL
SEEDING DATE: JANUARY 3, 1939
IRRADIATION DATE: GERMINATION TO HARVEST
HARVEST DATE: APRIL 21, 1939
NUMBER OF PLANTS PER TREATMENT: 12

TREATMENT	AV. HEIGHT	No. DAYS TO BUD	No. DAYS TO FLOWER
	cm.		
Short day	111*	74	98
Blue	113*	74	104
Blue 4X	114*	76	108
Yellow-green	121	.....	.....
Red	130	.....	.....

* Height to base of flowering spike.

TABLE XI

FLOWERING DATA ON *Tithonia speciosa*
SEEDING DATE: DECEMBER 5, 1938
IRRADIATION DATES: GERMINATION TO HARVEST
HARVEST DATE: MARCH 21, 1939
NUMBER OF PLANTS PER TREATMENT: 20

TREATMENT	NO. DAYS TO BUD	NO. DAYS TO FLOWER	AV. NUMBER OF FLOWERS AND BUDS
Short day	38	74	32 flowers and flower buds
Blue	51	78	28 flowers and flower buds
Blue 4X	56	86	21 flowers and flower buds
Yellow-green	.....	.....	15 vegetative buds
Red	.....	.....	18 vegetative buds

increased in dry matter only under red and yellow-green radiation and the most pronounced increases generally occurred under the influence of the longer wavelengths. The longer wavelengths caused a decrease in the percentage of dry matter in all the plants studied except Scabiosa, where there was little variation between any of the plots at the time of harvest. There was a definite decrease in the percentage of dry matter with the higher blue intensity with spinach, Xanthium and soybean.

The dry weight data as given in the tables for the blue plot is in several instances higher than the control and blue 4X plots. This is especially true of the aster and Scabiosa data. This condition was possibly caused by the slightly higher air temperature in the end of the greenhouse where the blue plot was located. The air temperature for this plot occasionally ran several

FIG. 2. *Callistephus chinensis*, variety Heart of France. Photographed after 71 days of irradiation.

FIG. 3. *Scabiosa atropurpurea*, variety Azure Fairy. Photographed after 81 days of irradiation.

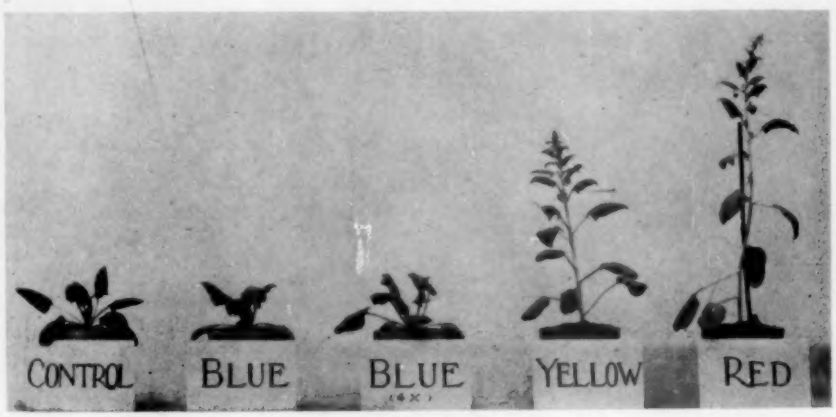

FIG. 4. *Spinacia oleracea*, variety Nobel. Photographed after 37 days of irradiation.

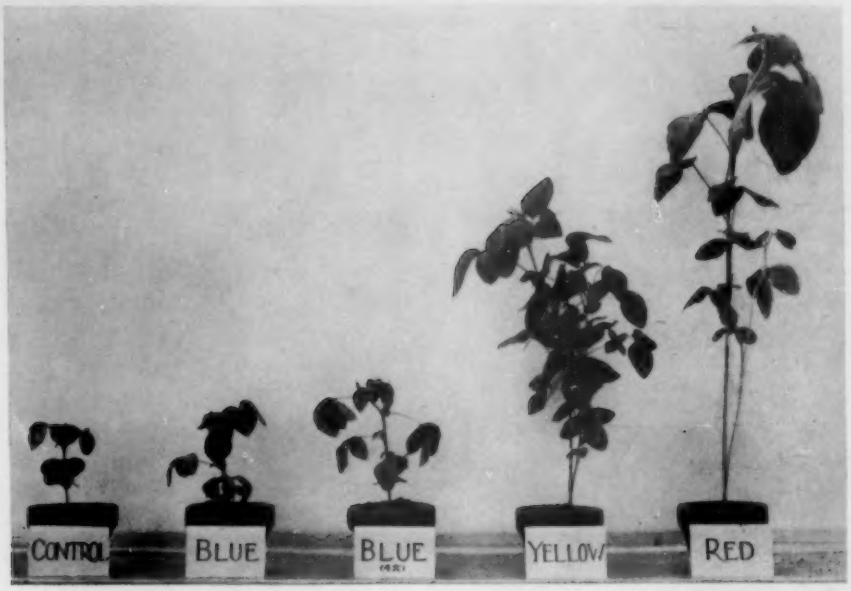

FIG. 5. *Soja max*, variety Mukden. Photographed 73 days after seeding.

degrees higher than that for the others owing to a door opening into an adjacent warmer house.

An increase in height occurred under the red radiation with all the plants used in the investigation. The greatest increase occurred with the long day plants. The aster and spinach in the short day treatment remained in rosette form, while the red and yellow-green radiation brought about marked increases in height; in the case of spinach this amounted to as much as 80 to 90 times. The relative increase in height in the short day plants was not so great, being of the order of from 0.1 to 3 times.

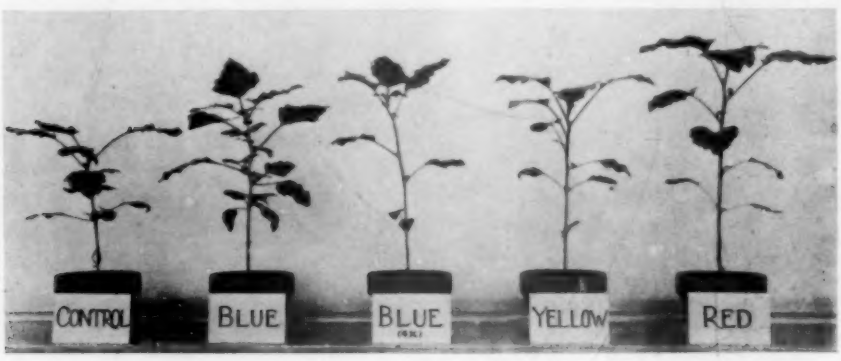

FIG. 6. *Xanthium pennsylvanicum*. Photographed 64 days after seeding.

The top-root ratio was increased under the red in all cases where weight data were taken, except with Xanthium where the ratio was highest under the short day treatment. With Xanthium, the decreased ratio appeared under the yellow-green and high intensity blue, as well as under the red. Because of this difference in relation to the other plants, a second group of these plants was grown under a 9-hour day and under a 9-hour day supplemented with 15 hours of red radiation. The data for these are presented in table IX. The red radiation again caused a lower top-root ratio than did the short day condition. In aster, spinach, and soybean the top-root ratio was increased by the yellow-green and blue radiation treatments, but not to the degree occurring under the red.

Aster showed a decreased number of leaves under the red, yellow-green and high intensity blue and a marked increase in leaf size, progressively through the blue, high intensity blue, yellow-green, and red. Leaf area also was increased to the greatest extent under the red, although all the supplemental radiations had some effect.

In soybean, there was no significant variation in number of leaves per plant among the various plots at the time of harvest, but there was an increase in leaf area under the supplemental light treatments in the same progressive order as for aster but the percentage increase was not so great as with aster.

In the case of Tithonia, it is interesting to note that the blue has an inhibitory effect on the number of flower buds, the high intensity blue having a greater inhibitory effect than the low.

Discussion

These results are quite consistent in indicating that it is the longer wavelengths of the visible spectrum which are chiefly responsible for the photoperiodic response of both long and short day plants to long day treatments. The red is apparently somewhat more effective than the yellow-green. The blue is only slightly influential in altering the response of the plant from that obtained in the short day condition. In general, height, weight, top-root ratio, and the percentage of dry matter all point to the conclusion that the longer wavelengths also induce the vegetative responses associated with the long day treatment.

Since the blue has little influence in both inhibition of flowering in short day plants and flower induction in long day plants, it appears that the blue radiation is not strongly absorbed by the photo-activated molecule involved in the photoperiodic system since only that radiation which is absorbed can be effective. Therefore, it must be assumed either that the absorbing molecule has strong absorption bands in the red, weaker bands in the yellow-green, and very weak bands in the blue, or that the blue bands are relatively

strong, but that a blue absorbing system such as the carotenoids is exhibiting a filtering effect. The latter hypothesis appears rather unlikely since, while the carotenoids are a part of the pigment system of the chloroplasts, they do not produce a sufficiently strong filtering effect to greatly reduce the absorption of blue radiation by the chlorophyll. Photosynthetic activity and chlorophyll absorption show maxima of the same order of magnitude in the blue and the red. It hardly seems likely that the carotenoids would exert any stronger filtering effect in the case of photoperiodic processes.

Assuming negligible filtering effect, the general region of maximum absorption within the visible spectrum of the photo-activated molecular species involved in the mechanism is in the red, with a somewhat decreased absorption in the yellow-green, and a greatly reduced absorption in the blue. The photo-activated molecule involved in photoperiodism is probably the same for both long and short day plants since the same general regions of radiation are effective in producing their respective responses.

Since the leaves of the plant have been identified as the principal loci for radiation absorption leading to photoperiodic flower induction and inhibition (**2, 6, 7**), it would appear that there is a pigment present in the leaves which has a region of maximum absorption in the red and a minimum in the blue. That this pigment must be present in all of these plants, regardless of their flowering response to daylength, is postulated from the results which show that the effective and therefore absorbed radiation for the plants investigated, both of the long and short day type, is in the longer wavelengths.

The results do not point beyond the initial absorption of radiant energy. It should be pointed out that the molecule absorbing the radiation is not necessarily involved in the series of steps leading to the eventual photoperiodic response further than the transfer of energy to activate other molecules. What type of reaction within the plants which leads to the induction of flower buds in the one case and the production of foliar buds in the other is not indicated.

The hypothesis presented herein as to the similar nature of the absorption reaction involved in the photoperiodic responses of long and short day plants is based upon results obtained with only three relatively wide bands of radiation of equal energy in the visible spectrum. Studies must be made at many more points in the visible spectrum with very narrow bands or monochromatic radiation before conclusive evidence can be presented as to the absorption spectrum of the photo-activated substance.

Summary

Three long day plants, *Callistephus chinensis*, variety Heart of France, *Scabiosa atropurpurea*, variety Azure Fairy, *Spinacia oleracea*, variety Nobel, and four short day plants, *Soja max*, variety Mukden, *Xanthium*

pennsylvanicum, *Salvia splendens*, variety Fairly Tall, and *Tithonia speciosa* were grown in subirrigation nutrient solution culture. They were given natural short winter days and days lengthened to 24 hours by blue radiation (4047 and 4358Å), yellow-green (5770 and 5791Å), and red (6400-9000Å) of approximately 100 ergs/cm.^{2}/second, and blue of 400 ergs/cm.^{2}/second.

All the long day plants set macroscopic flower buds under red and yellow-green radiation; spinach and Scabiosa failed to set buds under blue or the short day condition. China aster, which was grown into the longer spring days, flowered under a 12-hour daylength, the blue radiation having only a slight effect in reducing flowering time.

All the short day plants flowered under the short day condition and blue radiation, but failed to set buds under either red or yellow-green.

The red radiation brought about an increase in dry matter in all the plants studied. In some cases, the yellow-green and blue radiations also brought about an increase in total dry weight. Except with Scabiosa, the red and yellow-green caused a decrease in the percentage of dry matter, however. The higher blue radiation also caused a decreased percentage of dry matter with spinach, Xanthium, and soybean.

Height was increased under the red and yellow-green with all the plants, the increase being of much greater magnitude with the long day plants than with the short day ones.

Top-root ratio increased under the longer wavebands in all cases with the exception of Xanthium where a decrease in ratio was noted. With aster, spinach, and soybean, the ratio was increased also by the blue but not to the extent occurring under the red.

Leaf area for aster and soybean was increased progressively from the short day through the blue, high intensity blue, yellow-green, and red.

The results secured in this investigation indicate that it is the longer wavelengths of the visible spectrum which are primarily effective in producing the flowering and vegetative effects secured under long day treatments with both the long and short day plants used. The initial photo-chemical step appears to be similar for all the plants tested.

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LITERATURE CITED

1. Borthwick, H. A., and Parker, M. W. Photoperiodic perception in Biloxi soybeans. Bot. Gaz. **100**: 374–387. 1938.

2. CAJLACHJAN, M. H. On the mechanism of photoperiodic reaction. Compt. Rend. Acad. Sci. U.R.S.S. **1**: 89–93. 1936.
3. FUNKE, G. L. Proeven over photoperiodiciteit by verschillend gekleurd licht. Biol. Jaarboek Nat. Genoot. Dodonaea **3**: 225–261. 1936.
4. ————. Proeven over photoperiodiciteit by verschillend gekleurd licht. Biol. Jaarboek Nat. Genoot. Dodonaea **4**: 345–359. 1937.
5. ————. Proeven over photoperiodiciteit by verschillend gekleurd licht. Biol. Jaarboek Nat. Genoot. Dodonaea **5**: 404–424. 1938.
6. HAMNER, K. C., and BONNER, JAMES. Photoperiodism in relation to hormones as factors in floral initiation and development. Bot. Gaz. **100**: 388–431. 1938.
7. KNOTT, J. E. Effect of a localized photoperiod on spinach. Proc. Amer. Soc. Hort. Sci. **31**: 152–154. 1934.
8. RASUMOV, V. The significance of the quality of light in photoperiodical response. Bul. Appl. Bot., Gen. and Plant Breeding, III. Phys., Biochem. and Anat. Plants **3**: 217–251. 1933.
9. SCHAPPELLE, N. A. Effect of narrow ranges of wavelengths of radiant energy, and other factors, on the reproductive growth of long day and short day plants. New York (Cornell) Agr. Exp. Sta. Mem. **185**. 1936.
10. WITHROW, R. B. A photoelectric device for the rapid measurement of leaf area. Jour. Agr. Res. **50**: 637–643. 1935.
11. ————, and BENEDICT, H. M. Photoperiodic responses of certain greenhouse annuals as influenced by intensity and wavelength of artificial light used to lengthen the daily period. Plant Physiol. **11**: 225–249. 1936.
12. ————, and BIEBEL, J. P. Photoperiodic response of certain long and short day plants to filtered radiation applied as a supplement to daylight. Plant Physiol. **11**: 807–819. 1936.
13. ————, and ————. Nutrient solution methods of greenhouse crop production. Indiana Agr. Exp. Sta. Cir. **232**: 1–20. 1938.

EFFECTS OF ENVIRONMENTAL FACTORS ON OXIDIZING ENZYMES OF ROSE MALLOW SEEDS

KATHRYN STALEY

(WITH EIGHT FIGURES)

Introduction

The power of plant tissues to break up hydrogen peroxide into water and molecular oxygen was known long before catalase was recognized as an enzyme, discovered, and named by LOEW (**18**) in 1901. This discovery opened a new field for investigation and many studies were undertaken. The wide distribution of catalase in plant and animal tissue led LOEW to conclude that it might serve as a protective measure against the injurious protoplasmic effects of hydrogen peroxide and possibly other related compounds formed in respiration. DIXON (**8**) has shown that when purine bases are oxidized by molecular oxygen in the presence of xanthine-oxidase as a catalyst, the oxidase undergoes destruction during the course of the reaction owing to the hydrogen peroxide that is formed. He found that this destruction could be prevented by the addition of catalase. Pneumococci in contact with a free supply of oxygen produced hydrogen peroxide in sufficient amount to inhibit their growth, but if catalase were added the bacteria grew vigorously.

A close correlation between catalase activity and respiration was found in potatoes (**2**), in rice grains germinated under water (**22**), and in sweet corn (**3**). CROCKER and HARRINGTON (**6**) found this same correlation in the seeds of Johnson grass but not in Amaranthus. RHINE (**26**) using a number of different seeds concluded that catalase activity is a measure of the metabolic activity only when there is a rapid change in respiration. The absence of catalase activity in certain blue green algae (**14**) and in anaerobic bacteria (**20**) indicates that catalase is not essential to the respiration of all cells. MCLEOD and GORDON (**20**) consider it possible that catalase destroys some injurious substance such as H_2O_2 which is produced by aerobic organisms and by anaerobic organisms in the presence of oxygen; thus anaerobic organisms lacking catalase would be injured when exposed to oxygen.

A variation in catalase activity was observed by POPE (**24, 25**) in the different parts of the same barley plant. The relation of the catalase activity to physiological breakdown in Jonathan apples convinced NELLER (**23**) that catalase activity could be used as a true index of the rate of metabolic activity. HEINICKE (**15, 16**) concluded that catalase activity is a highly sensitive index to changes in the internal condition of the apple tree, and that this activity varies with the season, with the part of the tree tested, and with

various environmental factors. He believed that the catalase activity would eventually prove as good an indicator of metabolism as do the temperature and pulse rate in the human body.

The detailed nature of catalase has been carefully investigated. LOEW (**18**) recognized an insoluble and a soluble form. He thought the insoluble form, or alpha catalase, was a compound of the soluble form with a nucleoprotein. The soluble form, beta catalase, was considered an albumose which could be liberated by the action of a very dilute alkaline medium upon the insoluble form. APPLEMAN (**1**) was able to separate the two forms by the use of ordinary filter paper, but could not do so with a Chamberland-Pasteur filter. Approximately fifty per cent. of the total enzyme content passed through the filter paper, indicating that the two forms were present in about equal amounts.

For the chemical analysis of catalase, liver has served as the most convenient source of the catalase preparation. EULER and JOSEPHSON (**11**) state that no relationship exists between the activity of the preparation and the percentage of iron present. They analyzed two preparations which had a catalytic activity of 43,000 and 40,000 respectively, and found that the former contained 0.63 per cent. iron while the latter contained 0.15 per cent. ZEILE and HELLSTRÖM (**32**) found that a strong preparation of catalase obtained from horse liver had a distinct absorption spectrum corresponding to a hematin compound. Regardless of degree of purity they thought there was a strict proportionality between the catalytic activity and the concentration of the porphyrin-bound iron. Comparing the value calculated for hemin with that for catalase they found that in the catalase union the iron was decidedly more active than the usual porphyrin-bound iron. They concluded that the porphyrin-iron complex in catalase must be bound in a very specific way to the protein or some other colloidal carrier, which binding confers to it this unusual increase of activity. They also found that such factors as high temperature, alkali, acids, or the addition of small amounts of KCN or H_2S, which abolished or inhibited the catalase activity, also modified the absorption spectrum of the hematin. STERN (**27**) studying the activity of ferrous and ferric ions, hemin, and catalase, respectively, in decomposing H_2O_2, found that the binding of iron into porphyrin resulted in a greater catalytic activity than for iron alone; and a still greater activity was obtained when hemin was coupled with catalase protein. The absorption spectrum and pH observations led STERN (**27, 28**) to conclude that the parahematin compounds were closely related to natural catalase. It has been estimated that the diameter of a catalase particle is 5.46 mμ and that the molecular weight of catalase is approximately 68,900. This indicates that the molecules are similar in size to those of hemoglobin. Horse liver catalase has been split by dialysis into two inactive components, one of low molecular

weight (possibly hemin) and the other possibly protein, which, upon being mixed become active. TAUBER and KLEINER (**30**) concluded that catalase solutions could be digested by trypsin.

Crystalline catalase prepared from beef liver (**29**) coagulated upon heating and showed many other properties characteristic of proteins. The investigators report that the pyridine hemochromogen test was readily obtained with this preparation. It appears, then, that catalase is similar to peroxidase (**13**) and oxidase (**13, 31, 44**) in having an iron-porphyrin complex as the active group.

The catalase studies reported here were undertaken to determine the effect of environmental factors such as temperature, exposure to gases, and the submergence of seeds in water upon the activity of this iron porphyrin complex of imbibed seeds. Other oxidizing enzymes also were studied to obtain a more generalized idea of the effect of such conditions on the enzyme complexes associated with oxidation in the plant.

In biological oxidations, peroxidases are thought to transfer the oxygen, linked as a peroxide, to oxidizable substances. They cannot decompose H_2O_2 in the absence of an oxidizable substance. The oxidases render such matter more susceptible to reduction.

Materials and methods

Seeds of the Malvaceae are noted for their hardness and longevity. Two varieties of rose mallow, or Hibiscus, were selected: the variety Golden Bowl, and variety Dark Red. The seeds of the former are larger than those of the latter and are approximately twice as heavy; both are very erratic in germination because of their impervious seed coats. Seeds of the 1936 crop of Dark Red and of the 1934 and 1936 crop of Golden Bowl were obtained from Henry Dreer Seed Co., Philadelphia, Pa. Only a small quantity of the 1936 crop of Golden Bowl was available.

In order to secure greater uniformity in germination the seeds were subjected to the action of H_2SO_4 (sp. gr. 1.84) for 53 minutes, washed with sterile water, and the acid neutralized with lime water. They were placed in sterile water until they showed evidence of swelling, disinfected with 0.25 per cent. solution of uspulin for one hour, and thoroughly washed with sterile water. The usuplin treatment was inadequate for the seeds which were kept under water for 30 days, consequently, a more effective method of disinfection was used. This treatment consisted of soaking the seeds 4 hours in a 1 : 30 dilution of Zonite and rinsing in sterile water before placing them under the conditions of the experiment.

All glassware, cellucotton, filter paper, and water used for the experiments were sterilized in the autoclave at 15 lb. pressure for 30 min. on each of two successive days. A modified APPLEMAN (**1**) apparatus was used for

catalase determinations; the liberation of oxygen from neutralized Merck's hydrogen peroxide served as a measure of the catalase activity. Each bottle was standardized by permanganate titration before using, and the equivalent of 10 ml. was used for each determination. The tissue was ground for 5 min. in a mortar with clean sand, a small excess of $CaCO_3$, and a measured amount of distilled water. The material was then washed into the shaking bottle with the remainder of the water previously measured. The equivalent of 10 ml. of water was used for each determination. The bottle was fastened in the shaker in such a manner that the former was suspended in a water bath maintained at a temperature of 20° C. When the contents of the bottle were of the same temperature as the water bath, the two-holed stopper fitted with dropping funnel and delivery tube was adjusted to position, and 10 ml. of neutralized H_2O_2 was added 15 seconds before the shaking began. The levelling bulb was used to maintain pressure equilibrium. Readings were taken at 1, 2, 3, 5, and 10 minutes. The first four readings served as a check on the final reading and showed the rate at which the molecular oxygen was evolved. The results were corrected for standard conditions of temperature and pressure.

Simplified Bunzell tubes and a mechanical shaker were used for oxidase determinations. The tissues were ground for 5 min. in a mortar with a very small quantity of distilled water. The ground tissue was then washed into a small graduated calibrated cylinder. The volume was brought to 4 ml. and the contents thoroughly mixed. One ml. of this mixture was pipetted into the short arm, and 5 ml. of a 1 per cent. solution of pyrogallol into the larger arm, of the Bunzell tube; care was taken not to mix the two until the shaker was started. The top was adjusted to leave the contents open to the outside air, while the tube was fastened into the mechanical shaker and allowed to stand until temperature equilibrium had been established: The top was then turned to prevent contact with the outside air and the shaker, adjusted to make one hundred complete excursions per minute, set in motion. Readings were made at 15-minute intervals for the first hour and at thirty-minute intervals for the second hour. At the end of the first hour the manometers were opened and reset. The Bunzell tubes were calibrated, and the results corrected for 25 ml. volume and standard conditions of temperature and pressure.

Results

Temperature effects on catalase

Imbibed seeds of the two varieties tested were placed on moist cellucotton and filter paper in sterile Petri dishes. The seeds were subjected to temperatures of 8°, 20°, 37°, 42°, and 47° C. for a period of seven days. Catalase determinations in duplicate were made daily. Six seeds of variety Golden Bowl

and twelve seeds of Dark Red, respectively, were used in a determination. All results were corrected to standard conditions.

Seeds of the 1934 crop of Golden Bowl subjected to temperatures of 8°, 20°, and 27° C. show a decrease in catalase activity, followed by an increase until the food is exhausted, at which time the catalase decreases (table IA).

TABLE I

TEMPERATURE EFFECTS ON CATALASE ACTIVITY OF HIBISCUS SEEDS. MILLILITERS OF OXYGEN LIBERATED IN TEN MINUTES

	Days	Temperature					
		8° C.	20° C.	27° C.	37° C.	42° C.	47° C.
		ml.	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*
A	0	39.6	39.6	39.6	39.6	39.6	39.6
	1	41.2	32.1	34.4	40.2	30.2	0.9
	2	40.3	40.5	34.8	40.2	9.7	0.0
Golden Bowl	3	29.7	71.0	66.9	36.3	14.8	0.0
Six seeds of 1934 crop	4	33.6	52.8	36.0	31.5	4.5	0.0
	5	42.6	76.6	55.4	14.2	4.3	0.0
	6	44.6	82.8	79.0	6.0	1.9	0.0
	7	45.9	55.6	97.1	4.8	1.0	0.0
B	0	36.6	36.6	36.6	36.6	36.6	36.6
	1	40.3	40.9	35.9	29.3	15.6	0.5
	2	32.6	33.9	34.6	28.2	16.8	0.4
Golden Bowl	3	33.0	38.4	45.4	15.5	7.1	0.4
Six seeds of 1936 crop	4	29.3	42.7	45.6	14.1	5.7	0.3
	5	32.2	48.5	65.9	13.4	4.7	0.2
	6	28.3	59.5	109.6	13.5	2.2	0.1
	7	30.9	69.8	93.5	5.8	1.4	0.1
C	0	38.8	38.8	38.8	38.8	38.8	38.8
	1	26.6	19.4	20.6	22.4	27.0	0.0
	2	28.0	46.2	57.4	35.5	19.5	0.0
Dark Red	3	39.7	46.8	35.1	31.8	4.8	0.0
Twelve seeds of 1936 crop	4	23.9	71.7	97.5	38.4	12.9	0.0
	5	22.6	91.5	105.3	38.5	8.6	0.0
	6	19.2	97.6	104.7	33.1	6.4	0.0
	7	24.5	109.4	86.5	29.5	4.9	0.0

The drop in catalase content is slower in the seeds kept at 8° C. since it takes place on the fourth day; at 20° and 27° C. the depression occurs on the first day. At 37° C. there is noted a very slight increase on the first and second day (the depression may have come earlier than the 24-hour limit) followed by a gradual decrease in catalase content. The seeds at this temperature gave no visible signs of germination. At 42° C. there is a slight decrease on the first day followed by a rapid drop in catalase activity. At 47° C. the decrease the first day is very rapid and soon reaches zero.

The catalase activity of the 1936 crop of variety Golden Bowl agrees rather closely with that of the 1934 crop (table IB). At 47° C. the catalase activity shows a slightly greater resistance to this temperature than that

of the 1934 crop. The results obtained indicate that the difference in age in these particular seeds is of no significance in catalase activities.

The seeds of variety Dark Red and Golden Bowl respond in a similar fashion in both germination and catalase activity (table IC, fig. 1, 2).

FIG. 1. The effect of temperature on the catalase activity of imbibed seeds of Hibiscus variety Dark Red. (a) 27° C., and (b) 8° C.

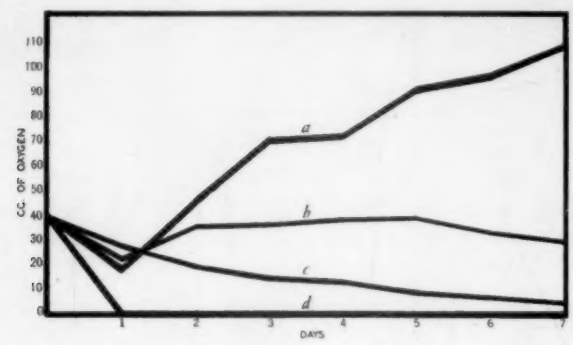

FIG. 2. The effect of temperature on the catalase activity of imbibed seeds of Hibiscus variety Dark Red. (a) 20° C., (b) 37° C., (c) 42° C., and (d) 47° C.

At 8°, 20°, 27°, and 37° C. there is a characteristic decline in catalase activity in the early stages of germination, followed by an increase. This decrease occurs later with seeds kept at 8° C. than with those at the higher temperatures. Good germination occurred at 20° and 27° C., while only a few were able to germinate at 37° C. Apparently this accounts for the greater activity of the catalase at 37° C. as compared with that of the Golden Bowl seeds at the same temperature. At 42° C. the seeds gave no indication of germination and there was a gradual decrease in the catalase activity. At 47° C. the catalase was completely inactivated during the first day.

The results indicate that the catalase activity follows a definite course. In the early stages of germination the activity is depressed, but increases as germination progresses, until the food supply is apparently exhausted.

The effect of oxygen and nitrogen on catalase

In order to determine the effect of oxygen and nitrogen on the catalase activity of imbibed seeds, swollen seeds of both species were placed on moist cellucotton and filter paper in a series of 8-oz. bottles. Each bottle contained a number of seeds sufficient for seven daily determinations. The bottles were connected and one series subjected to a steady stream of oxygen; the second series was subjected to a steady stream of nitrogen. The bottles were kept in the basement at a room temperature of 25°–27° C. Six seeds of Golden Bowl and twelve seeds of Dark Red, (except where indicated) were used in each determination.

TABLE II

Catalase activity of Hibiscus seeds treated with oxygen. Milliliters of oxygen liberated in minutes on seven consecutive days

Days	Minutes									
	Six seeds of Golden Bowl					Twelve seeds of Dark Red				
	1	2	3	5	10	1	2	3	5	10
	ml.	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*
0	6.7	14.6	20.7	23.0	39.6	9.2	14.8	19.4	26.8	38.8
1	5.7	11.8	15.7	20.6	30.4	7.3	11.8	17.1	23.8	34.0
2	10.9	16.9	21.4	28.1	37.6	13.1	22.0	33.3	45.0	69.1
3	12.1	20.9	28.3	39.1	57.1	21.4	35.4	46.9	64.1	89.7
4	11.7	20.6	27.6	38.9	57.8	35.3	60.4	79.0	108.4	152.9*
5	15.1	31.7	36.0	52.6	75.1	34.9	58.1	78.6	109.5	160.8*
6	17.6	28.3	36.9	51.9	76.5	30.1	49.7	64.4	88.7	127.4*
7	6.1	9.9	14.1	20.9	33.7	30.0	49.0	62.9	86.6	118.3*

* Owing to the high catalase activity, 6 seeds were used for each determination and four determinations made so that the number given above is the amount of O_2 evolved by 12 seeds.

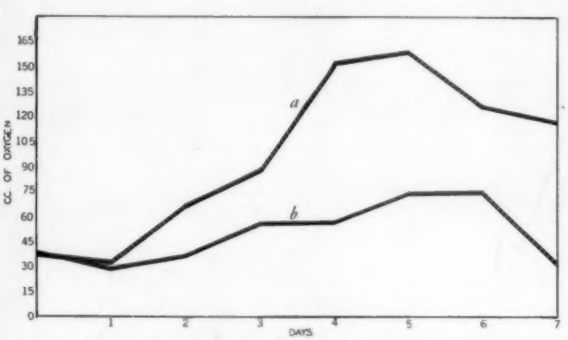

Fig. 3. The effect of oxygen on the catalase activity of imbibed seeds of (a) variety Dark Red and (b) variety Golden Bowl. Based on 10-minute data.

The results given in table II and figure 3 are for 1, 2, 3, 5, and 10 minutes. This shows the rate at which the oxygen is evolved as well as the final reading. The results given indicate that the seeds of the two species treated with oxygen show the same general course of catalase activity which is similar to that obtained in the seeds subjected to temperatures favorable for germination. The seeds of both species show a decrease in catalase on the first day, with an increase reaching the peak for variety Dark Red on the 5th day and for Golden Bowl on the 6th day, followed by a decrease. The conditions were favorable for good germination.

The results of the treatment with nitrogen are essentially the same as those obtained with oxygen (table III, fig. 4). The amount of increase in

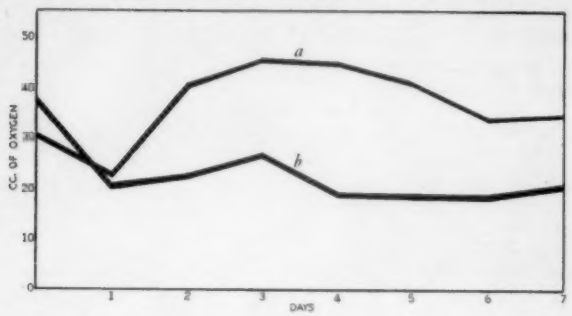

FIG. 4. The effect of nitrogen on the catalase activity of imbibed seeds (a) Golden Bowl and (b) Dark Red. Based on 10-minute data.

catalase activity is less, however, and it was not completely inactivated during the seven day period. Only the initial stages of germination were noted since the absence of oxygen precluded the possibility of the usual reaction.

TABLE III

CATALASE ACTIVITY OF HIBISCUS SEEDS TREATED WITH NITROGEN. MILLILITERS OF OXYGEN LIBERATED IN MINUTES ON SEVEN CONSECUTIVE DAYS

DAYS	MINUTES									
	SIX SEEDS OF GOLDEN BOWL					TWELVE SEEDS OF DARK RED				
	1	2	3	5	10	1	2	3	5	10
	ml.	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*
0	6.7	14.7	20.7	23.0	31.0	9.2	14.8	19.4	26.8	38.8
1	4.9	10.3	13.6	19.0	23.8	4.9	8.4	11.6	15.6	21.3
2	11.0	18.7	25.0	32.5	40.8	5.1	8.7	12.2	16.7	23.1
3	12.1	20.6	27.2	35.6	45.5	5.2	9.5	13.1	18.8	27.3
4	6.8	14.7	19.6	29.6	45.3	4.5	7.7	10.3	14.1	19.4
5	7.6	14.8	21.3	33.7	40.9	4.0	6.6	9.6	13.6	19.2
6	7.2	13.2	18.4	25.5	34.4	3.7	6.7	9.3	13.6	19.4
7	6.9	11.7	16.4	23.1	35.0	3.6	6.7	9.4	15.3	20.8

Catalase activity of submerged seeds

Imbibed seeds were placed in a series of plugged tubes containing sterile distilled water to a depth of 5, 100, and 155 mm. The seeds were kept at room temperature for a period of thirty days and catalase determinations were made daily. Six seeds of Golden Bowl and 12 seeds of Dark Red were used in the determinations. Under all conditions there was a decrease in catalase activity in the early part of the time period then a slight increase followed by a marked decrease (table IV). Figure 5 shows the course of the catalase activity of the 1934 crop of Golden Bowl at the various depths. The embryos were able to undergo the early stages of germination and a few pushed the radicle through the seed coat. The rate and amount of decrease

TABLE IV

Catalase activity of Hibiscus seeds submerged in water. Milliliters of oxygen liberated in ten minutes on thirty consecutive days

Days	Golden bowl, 6 seeds						Dark Red, 12 seeds		
	1934 crop			1936 crop			1936 crop		
	Water depth			Water depth			Water depth		
	5 mm.	100 mm.	155 mm.	5 mm.	100 mm.	155 mm.	5 mm.	100 mm.	155 mm.
	ml.	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*
0	45.0	45.0	45.0	47.0	47.0	47.0	39.0	39.0	39.0
1	35.6	44.4	28.3	30.8	40.4	37.7	22.6	13.8	21.5
2	45.7	37.3	39.7	36.1	39.6	41.7	25.0	23.0	23.7
3	44.5	46.6	50.0	43.4	40.4	29.6	19.7	20.7	22.2
4	42.8	50.5	41.7	41.7	40.5	42.4	30.4	20.9	21.9
5	42.1	43.2	56.5	34.5	39.2	37.6	26.9	33.6	24.9
6	53.3	41.9	42.1	27.6	43.7	37.5	21.5	25.4	19.3
7	43.6	37.4	43.1	43.6	41.7	39.7	22.8	16.3	18.2
8	33.6	33.9	36.3	43.6	34.5	32.9	23.2	27.5	21.3
9	27.5	19.6	44.0	48.5	33.2	38.6	22.7	20.2	21.1
10	31.5	32.8	43.9	44.7	36.3	40.1	20.9	20.2	17.4
11	34.4	19.9	22.9	44.1	34.5	41.3	21.9	15.8	22.8
12	39.8	18.2	16.4	48.3	38.3	34.9	21.9	14.6	18.1
13	33.7	18.0	19.8	44.9	33.8	34.1	20.8	14.6	19.2
14	33.8	18.5	23.9	38.2	33.7	37.1	21.8	12.1	15.9
15	41.6	13.4	19.6	36.2	30.0	30.5	20.3	12.3	15.1
16	37.7	13.5	8.8	39.9	16.9	26.7	21.1	23.9	16.2
17	25.2	15.5	9.8	40.1	13.8	21.7	16.5	19.5	15.4
18	21.4	18.4	9.0	42.2	12.2	18.1	27.2	16.9	17.3
19	18.9	15.5	10.1	27.9	18.6	11.9	25.1	11.8	21.4
20	22.1	9.4	13.6	27.7	17.4	3.2	24.4	12.2	18.2
21	20.4	13.4	9.9	30.0	19.2	6.9	20.7	22.4	13.8
22	18.9	14.1	5.5	32.8	23.8	10.2	18.5	9.2	12.9
23	27.2	14.4	1.4	32.1	29.7	7.1	18.4	15.6	9.0
24	16.8	14.3	2.9	31.6	19.9	3.3	17.3	14.6	12.2
25	16.3	12.2	2.3	25.0	17.4	6.6	20.7	15.3	15.1
26	17.5	10.4	3.6	15.7	3.6	5.3	21.2	13.5	14.6
27	17.8	11.3	3.7	27.9	5.8	3.4	19.6	13.6	9.4
28	8.3	7.3	2.6	27.1	4.1	2.0	26.8	10.3	13.7
29	13.6	4.8	0.3	28.9	2.9	1.7	19.4	7.6	17.5
30	13.4	0.7	0.2	21.1	1.0	0.2	21.7	14.8	16.6

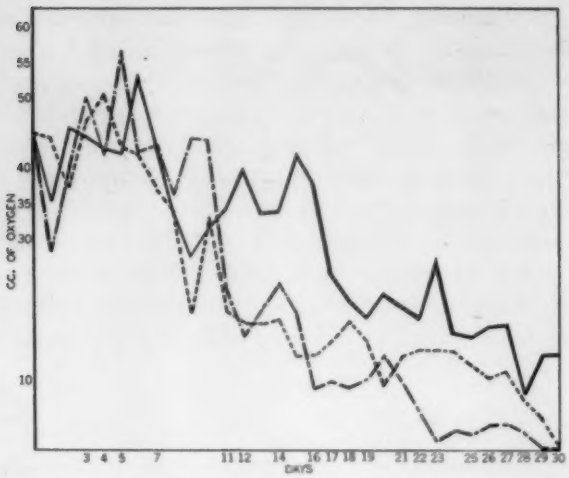

FIG. 5. The effect of a limited supply of oxygen on the catalase activity of Golden Bowl, 1934 crop. Solid line represents imbibed seeds immersed under 5 mm. of water, line of short dashes seeds immersed under 100 mm., and line of long and short dashes seeds under 155 mm.

was evidently correlated with the oxygen supply. The seeds submerged at a depth of 100 mm. and 155 mm. showed a much greater decrease of catalase activity over the later period of the experiment than did those at a depth of 5 mm. In all cases the decline in the catalase activity was well under way in the seeds subjected to 100 mm. and 155 mm. by the fifteenth day. The Golden Bowl seeds of the 1934 crop, at all three depths, showed at some stage in the course of the experiment a greater catalase activity than was present at the beginning. This was shown by the seeds of the 1936 crop of the same species at the 5-mm. depth and not at all by the seeds of variety Dark Red.

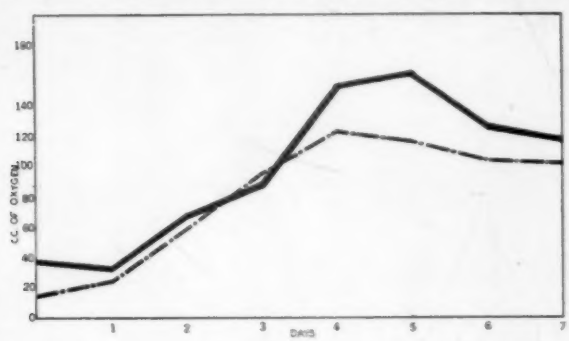

FIG. 6. The catalase activity of seeds of variety Dark Red treated with oxygen: solid line represents imbibed seeds, and the broken line imbibed and held under 155 mm. of water for 30 days before testing.

At the end of the thirty-day period the seeds of this variety showed a greater catalase activity than did those of Golden Bowl.

Seeds of variety Dark Red after being submerged thirty days under a 155-mm. layer of water were removed and placed under ideal germinating conditions with a steady stream of oxygen passing over them; the seeds thus treated gave a 50 per cent. germination. Catalase determinations of the germinating seeds were made on seven consecutive days. Figure 6 shows a comparison of the catalase activity of seeds submerged for 30 days, and of imbibed seeds when placed under oxygen. The submerged seeds show an increase in catalase activity and follow the same general pattern as do the imbibed seeds.

Temperature effects on oxidase

Imbibed seeds were placed on moist filter paper and cellucotton in sterile Petri dishes and subjected to 8° and 47° C. for a period of 7 days. Oxidase determinations were made daily using 12 seeds of the Golden Bowl and 24 seeds of Dark Red. The results show (table V) that oxidase, like catalase, is destroyed or inactivated at 47° C. The amount of oxidase fluctuates at 8° C., showing no great increase, and reaches its lowest level on the fourth day in the seeds of Golden Bowl and on the seventh day in those of Dark Red.

TABLE V

Temperature effects on oxidase activity of Hibiscus seeds. Manometer readings expressed in centimeters of mercury for two hours on seven consecutive days

Days	Golden Bowl, 12 seeds		Dark Red, 24 seeds	
	8° C.	47° C.	8° C.	47° C.
	cm.	*cm.*	*cm.*	*cm.*
0	−0.51	−0.51	−0.49	−0.49
1	−0.25	−0.00	−0.27	−0.00
2	−0.41	−0.00	−0.21	−0.00
3	−0.21	−0.00	−0.33	−0.00
4	−0.14	−0.00	−0.29	−0.00
5	−0.20	−0.00	−0.13	−0.00
6	−0.19	−0.00	−0.15	−0.00
7	−0.33	−0.00	−0.07	−0.00

Effect of oxygen and nitrogen on oxidase

The imbibed seeds were placed in bottles in the same manner as in the catalase experiment and subjected to identical conditions. Daily determinations were made using 12 of the larger variety and 24 seeds of the smaller variety as in the temperature tests.

The seeds subjected to the steady stream of oxygen showed rapid germination, but the oxidase content seemed to fluctuate during the early part of the experiment. The oxidase activity reached its maximum degree in

Golden Bowl seeds on the 6th day and in the seeds of Dark Red on the 5th day. The results indicate that the oxidase content does vary with germination of the seeds.

TABLE VI

OXYGEN EFFECTS ON OXIDASE ACTIVITY OF HIBISCUS SEEDS. MANOMETER READINGS EXPRESSED IN CENTIMETERS OF MERCURY FOR TWO HOURS ON SEVEN CONSECUTIVE DAYS

SEEDS	DAYS							
	0	1	2	3	4	5	6	7
	cm.	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
Golden Bowl (12)	−0.51	−0.22	−0.37	−0.41	−0.30	−0.33	−0.72	−0.37
Dark Red (24)	−0.49	−0.24	−0.36	−0.24	−0.52	−0.92	−0.68	−0.61

Only a few of the Golden Bowl seeds showed any visible signs of germination when subjected to nitrogen and the oxidase content, or activity, showed great fluctuation. The seeds of Golden Bowl showed a greater oxidase activity on the 5th day than did those treated with oxygen; this was not true for variety Dark Red although the high point occurred on the 5th day as it did in the oxygen-treated seeds.

TABLE VII

NITROGEN EFFECTS ON OXIDASE ACTIVITY OF HIBISCUS SEEDS. MANOMETER READING EXPRESSED IN CENTIMETERS OF MERCURY FOR TWO HOURS ON SEVEN CONSECUTIVE DAYS

SEEDS	DAYS							
	0	1	2	3	4	5	6	7
	cm.	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
Golden Bowl (12)	−0.51	−0.23	−0.67	−0.15	−0.75	−0.94	−0.41	−0.41
Dark Red (24)	−0.49	−0.16	−0.44	−0.24	−0.16	−0.60	−0.52	−0.56

THE OXIDASE ACTIVITY OF SUBMERGED SEEDS

Imbibed seeds were placed in tubes containing sterile water as in the catalase experiment and under identical conditions. Twelve seeds were used for the daily oxidase determination of Golden Bowl and 24 seeds for the other species. In both varieties the first part of the period was marked by considerable fluctuation of oxidase activity in the seeds at all three depths. After the 15th day the oxidase activity decreased consistently.

TABLE VIII

OXIDASE ACTIVITY OF HIBISCUS SEEDS SUBMERGED IN WATER. MANOMETER READINGS EXPRESSED IN CENTIMETERS OF MERCURY FOR TWO HOURS ON THIRTY CONSECUTIVE DAYS

Days	Golden Bowl, 12 seeds			Dark Red, 24 seeds		
	1934 crop			1936 crop		
	Water depth			Water depth		
	5 mm.	100 mm.	155 mm.	5 mm.	100 mm.	155 mm.
	cm.	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
0	−0.51	−0.51	−0.51	−0.49	−0.49	−0.49
1	−0.48	−0.67	−0.71	−0.75	−0.46	−0.63
2	−0.41	−0.31	−0.37	−0.83	−0.10	−0.23
3	−0.42	−0.64	−0.41	−0.41	−0.06	−0.39
4	−0.30	−0.25	−0.30	−0.79	−0.51	−0.35
5	−0.25	−0.57	−0.25	−0.41	−0.25	−0.35
6	−0.34	−0.24	−0.22	−0.75	−0.61	−0.27
7	−0.30	−0.40	−0.28	−1.13	−0.25	−0.35
8	−0.64	−0.40	−0.22	−1.00	−0.51	−0.46
9	−0.68	−0.16	−0.18	−0.67	−0.67	−0.27
10	−0.71	−0.28	−0.17	−1.34	−0.58	−0.55
11	−0.47	−0.36	−0.14	−0.54	−0.32	−0.59
12	−0.41	−0.24	−0.14	−0.58	−0.34	−0.59
13	−0.52	−0.12	−0.07	−0.40	−0.35	−0.43
14	−0.23	−0.20	−0.17	−0.52	−0.25	−0.07
15	−0.31	−0.36	−0.14	−0.66	−0.19	−0.23
16	−0.20	−0.24	−0.17	−0.54	−0.22	−0.15
17	−0.15	−0.24	−0.17	−0.33	−0.18	−0.15
18	−0.11	−0.28	−0.18	−0.41	−0.29	−0.23
19	−0.22	−0.24	−0.18	−0.66	−0.26	−0.17
20	−0.19	−0.16	−0.17	−0.54	−0.09	−0.15
21	−0.22	−0.24	−0.17	−0.54	−0.15	−0.15
22	−0.27	−0.29	−0.15	−0.58	−0.00	−0.07
23	−0.42	−0.12	−0.14	−0.37	−0.00	−0.15
24	−0.22	−0.16	−0.11	−0.41	−0.18	−0.27
25	−0.29	−0.16	−0.14	−0.16	−0.10	−0.11
26	−0.11	−0.28	−0.14	−0.16	−0.19	−0.11
27	−0.21	−0.08	−0.14	−0.46	−0.28	−0.19
28	−0.19	−0.16	−0.14	−0.50	−0.11	−0.15
29	−0.20	−0.28	−0.14	−0.62	−0.12	−0.15
30	−0.36	−0.24	−0.12	−0.75	−0.19	−0.15

The seeds at the greater depth showed a greater decrease than did those at the 5 mm. depth. Some of the submerged seeds were able to push the radicle through the seed coat. Where the oxidase is present in small amounts, this lack of uniformity in the degree of germination in the individual seeds may be responsible for some of the fluctuation if the oxidase content is influenced by germination as the results indicate (table VI, fig. 7).

Discussion

Catalase activity in the seeds of Hibiscus varieties Golden Bowl and Dark Red is depressed in the early stage of germination and then increases until

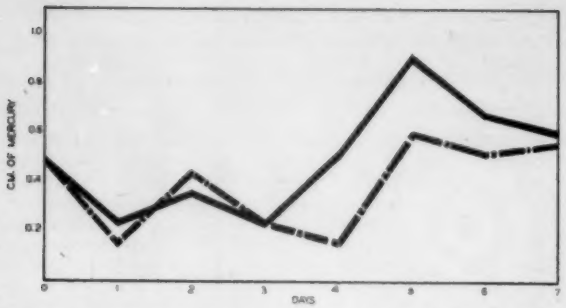

FIG. 7. The oxidase activity of seeds of variety Dark Red: solid line represents the imbibed seeds treated with oxygen, and the broken line seeds treated with nitrogen. The pressures are negative.

the food supply is apparently exhausted. This depression in the catalase activity is not in accordance with the findings of CROCKER and HARRINGTON (**6**) for grass seeds, but does agree with the work of RHINE (**26**) for wheat, feterita, clover, mustard, radish, and buckwheat, and of LANTZ (**17**) for corn. RHINE concluded that catalase increased with the need of the plant to destroy the injurious substances produced in respiration, and thus these by-products stimulated the production of catalase. She explained this decrease in the first stages of germination by assuming that the catalase reserve was used up, in attacking the respiratory products, faster than it

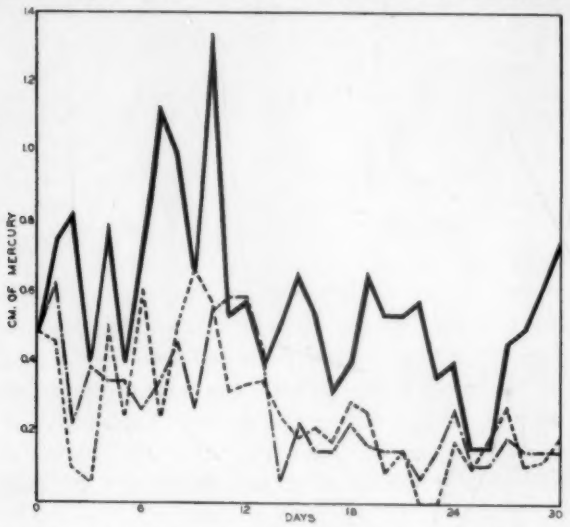

FIG. 8. The effect of a limited supply of oxygen on the oxidase content of imbibed seeds of variety Dark Red; solid line represents seeds immersed under 5 mm. of water, line of short dashes 100 mm., and line of long and short dashes 155 mm. The pressures are negative.

was produced. The increased stimulation of the accumulated respiratory by-products would, in a very short time, result in an increased production of catalase. The time required by the Hibiscus seeds to reach this low point of catalase activity depends on the speed of germination which in turn is dependent upon the external and internal conditions of the seeds.

The effect of the external conditions on the speed and the amount of catalase activity in the Hibiscus seeds is shown in the temperature studies in table I and figures 1 and 2. The seeds subjected to 8° C. required a longer period for the decrease in the catalase activity to occur than did those at 20° and 27° C. At the end of the seven-day period at this low temperature there was no evidence of the radicles pushing through the seed coats. The rate of increase of the catalase after the lowest point is reached is very slow in contrast to that in the actively germinating seeds. The lowest point of the catalase activity in the seeds placed at 20° and 27° C. is reached on the second or third day; there is then a sharp increase, reaching the peak during the 5th to 7th day. There is a slight difference in the catalase activity of the two species at 37° C. Variety Dark Red shows a decrease on the second day, with a very slight increase from the 2nd through the 5th, then a gradual decline. A few of these seeds were able to push the radicle through the seed coat at this temperature. The catalase activity of the seeds of Golden Bowl either reached the early decrease before the end of 24 hours or the seeds were unable to germinate at this temperature. At 47° C. in both varieties of seeds there is an abrupt drop in the catalase content beginning with the first day, while at 42° C. the trend is downward but at a slower rate.

The decrease and the lack of increase at the higher temperatures may be attributable to a combined set of factors. At 42° and 47° C. there is no evidence of germination, and since there seems to be some correlation between the germination and rate of catalase activity, this may be responsible for the absence of an increase in catalase activity. On the other hand, catalase shows its similarity to other enzymes in its reaction to several external conditions. There is no one temperature at which inactivation of all enzymes takes place, yet the temperature, time of exposure to heat, and the presence of other substances seem to play an important part in the rate of inactivation (**12, 21**). MORGULIS and BEBER (**21**) state that catalase is slowly inactivated at temperatures below 50° C.; inactivation becomes very rapid as the temperature is raised to 55° C. or above, especially at a pH of less than 6. Considering enzymes in general, FALK (**12**) finds that they show their greatest catalytic activity at temperatures near 40° C. At higher temperatures he thinks it is probable that inactivation of the enzymes takes place with sufficient rapidity to cause an apparent decrease in catalytic action. Investigators using the catalase extract have been able

to subject it to a higher temperature for a short period of time without completely destroying the catalytic activity. This shows the importance of the length of time the enzyme is exposed to a given temperature. An increase in the catalytic activity of a yeast suspension was obtained by EULER and BLIX (**10**) by heating for one hour at a temperature of 55°–60° C. They attributed this increased activity to the destruction of an inhibitory substance. CROCKER and HARRINGTON (**6**) heated air dry seeds of *Amaranthus retroflexus* for 4 hours at a temperature of 81° C. with a loss of only 40 per cent. of its original catalase activity, while the vitality of the seed was reduced 75 per cent. They found the reverse to be true for the seeds of Johnson grass. Thus the temperature which will inactivate catalase is determined by the source of the catalase and the conditions under which it is exposed.

The Hibiscus seed subjected to a steady flow of oxygen germinated rapidly at 25°–27° C. and the catalase activity, after decreasing for a short period, increased rapidly as is shown in table II and figure 3. Submerged seeds with a limited supply of oxygen showed similar reaction but there is a decrease in the amount of catalase activity (table IV, fig. 4). This decrease is greater in the seeds submerged at depths of 100 and 155 mm. than at 5 mm. When the oxygen is completely eliminated by passing a steady stream of nitrogen (table III, fig. 5) over the imbibed seeds there is a decrease in the catalase activity of the seeds of Dark Red; in Golden Bowl there is first a decrease, then a slight increase, and finally a second decrease which is typical of germinating seeds. A few of the Golden Bowl embryos were able to push the radicle through the seed coat; none of the Dark Red embryos was able to do so. MARKS and FOX (**19**) found that catalase extract obtained from muscle, stored under nitrogen, was inactivated at a slower rate than when kept under oxygen or air. It is probable that the effect on the increase of catalase activity is indirect, since germination proceeds rapidly when the seeds are exposed to air or excess oxygen and favorable temperature conditions, while the germination is depressed when the seeds are subjected to a very limited supply of oxygen or none at all. The amount of decrease found over a period of 30 days may be caused by lack of oxygen. When seeds of variety Dark Red were kept under 155 mm. of water for 30 days, then removed and subjected to a steady stream of oxygen and favorable germinating conditions, 50 per cent. of the seeds germinated and the catalase activity of the germinating seeds increased as shown in figure 6. The increase follows the same general course as for imbibed seeds. MORINAGA (**22**) found that in rice germinated aerobically there was an increase in catalase while in anaerobic germination there was a decrease in the amount of catalase and also in the length of the radicle.

Catalase activity in germinating seeds decreases when the food supply is exhausted according to the findings of LANTZ (17) and DELEANO (7). This is shown to be the case in the Hibiscus seeds. CROCKER and HARRINGTON (6) found that the catalase activity of the embryo of Stoner wheat and Sudan grass was 28 to 29 times that of the endosperm. They explain this by claiming that the endosperm is composed mainly of dead cells with the exception of the aleurone layer. HEINICKE (16) cites the work of AUCHTER and others in which a correlation was found between the nitrogen content of the tissues and its ability to destroy H_2O_2, while an increase in carbohydrate content without further addition of nitrogen depresses catalase. HEINICKE (15, 16) noted that the catalase activity in apple-leaf tissue was influenced by the factors which affected the nutritive or physiological conditions. He found that nitrogenous fertilizers increased the catalase activity of leaves from trees growing in sod. Bark of the roots had a higher activity than did bark from the trunk and the bud tissues were the most active of any on the tree. Strains of corn high in oil content displayed the greatest catalase activity, and the high protein strains ranked second when compared with low fat and low protein strains, according to LANTZ (17). BILETZKY (4) contends that more attention should be given to the factors concerned in the production of the plant and their relation to the catalase content. His findings correspond with others who state that nitrogen fertilizer tends to increase the catalase content. Since catalase apparently increases through the stages in germination when the stored food material is being converted into soluble form, with a subsequent decrease when this material is exhausted, the formation of the catalase may be brought about by the breaking up of the stored food. DOYLE (9) states that there is a temptation to look upon catalase as a labile metabolic by-product rather than as an enzyme with a fundamental physiological function.

The oxidases in general were found to react to the various environmental conditions in much the same way as did the catalase. The amount of oxidase present in the seeds before treatment was negligible, consequently it was necessary to increase the number of seeds used for the determinations. In a seed with such an impervious seed coat oxidation is doubtless reduced to a minimum; thus the imbibed seeds have a greater oxidase content than do the dry seeds. The oxidase increased in the seeds which were able to germinate. CROCKER and HARRINGTON (6) found a greater oxidase activity in coleoptiles and roots than in the whole seeds of Johnson grass, but believed the ratio was no greater than would be expected from the comparison of embryo ends with caryopses.

Oxidase was similar to catalase in its response to low and high temperatures. The oxidases were inactivated at 47° C. by the end of a 24-hour

period. At 8° C. there was no great increase in the amount of oxidase over a period of 7 days.

The seeds subjected to an abundant supply of oxygen showed considerable fluctuation in the oxidase activity during the first few days but reached a very high peak on the 5th day for variety Dark Red and on the 6th day for Golden Bowl. The irregularities found during the first part of the period would undoubtedly be smoothed out with a greater number of determinations. In general, the oxidase increased during the period of germination. When the oxygen supply is limited, or lacking completely, there is a remarkable amount of fluctuation in the oxidase content. The results (table VIII) indicate a greater difference in the oxidase content in seeds of variety Dark Red than in those of Golden Bowl when the supply of oxygen is limited. At the shallower submerged depth, seeds of variety Dark Red showed a much higher oxidase content than when kept at greater depths, and at all depths oxidase decreased considerably after the 11th or 12th day. When the supply of oxygen was replaced by nitrogen the Golden Bowl seeds showed a greater power of germination and greater oxidase activity than did the seeds of variety Dark Red.

Summary

1. The effect of temperature, an excessive supply of oxygen, a limited supply of oxygen, and the absence of oxygen on the catalase and oxidase activity of imbibed seeds of two varieties of Hibiscus, or rose mallow, was studied.

2. In all seeds capable of germination the catalase activity follows a very definite course. In the earliest stages of germination the catalase activity is depressed but soon increases rapidly as germination progresses. The oxidase content shows some fluctuation at first, then increases according to the rate of germination.

3. Temperature plays an important rôle in the germination of the seeds and in the activity of the enzymes. At low temperatures (8° C.) germination is slowed up as is catalase and oxidase activity. Medium temperatures (20°–27° C.) favor germination and likewise favor increased activity of oxidase and catalase. Temperatures above 40° C. inhibit germination of the seeds; correspondingly the enzymes not only fail to increase but decrease very rapidly.

4. An excess or adequate supply of oxygen favors germination of the seeds and also favors an increase in the activity of oxidase and catalase. When the supply is limited or inadequate there is a tendency for germination to be retarded or inhibited and this is accompanied by corresponding loss of activity of the enzymes.

5. There is a close relationship between the environmental factors and the activity of the oxidizing enzymes in rose mallow seeds.

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LITERATURE CITED

1. APPLEMAN, C. O. Some observations on catalase. Bot. Gaz. **50**: 182–192. 1910.
2. ————————. Relation of oxidases and catalases to respiration in plants. Amer. Jour. Bot. **3**: 223–233. 1916.
3. ————————. Respiration and catalase activity in sweet corn. Amer. Jour. Bot. **5**: 207–209. 1918.
4. BILETZKY, M. Über die Abhängigkeit der Katalaseaktivität der Pflanzen von den Ernährungs- und Wachstumsbedingungen. Fermentforsch. **13**: 467–498. 1933.
5. BUNZELL, HERBERT H. The measurement of the oxidase content of plant juices. U. S. D. A. Bur. Plant Ind. Bull. 238. 1912.
6. CROCKER, WM. and HARRINGTON, G. T. Catalase and oxidase content of seeds in relation to their dormancy, age, vitality and respiration. Jour. Agr. Res. **15**: 137–174. 1918.
7. DELEANO, N. T. Recherches chimiques sur la germination. Centralbl. Bakt. II, **24**: 130–146. 1909.
8. DIXON, M. Studies of xanthine oxidase. V. The function of catalase. Biochem. Jour. **19**: 507–512. 1925.
9. DOYLE, J. and CONNOR, P. O. Seasonal change in catalase content of conifer leaves. Ann. Bot. **44**: 907–915. 1930.
10. EULER, H. and BLIX, R. Verstürkung der Katalasewirkung in Hefezellen. Zeitschr. physiol. Chem. **105**: 83–114. 1919.
11. EULER, N. v. and JOSEPHSON, K. Über Katalase I. Ann. **452**: 158–181. 1927.
12. FALK, G. K. Chemistry of enzyme actions. Chemical Catalog Co. Inc. N. Y. 1924.
13. HAND, D. B. Peroxidase: A comparison with other iron porphyrin catalysts in cells. Ergebn. Enzymforsch. **2**: 272–281. 1933.
14. HARVEY, R. B. Enzymes of thermal algae. Science n.s. **60**: 481–482. 1924.
15. HEINICKE, A. J. Catalase activity as an indicator of the nutritive condition of fruit tissues. Proc. Amer. Soc. Hort. Sci. **19**: 209–214. 1922.

16. ————————. Seasonal variation in the nutrient condition of apple trees as indicated by catalase activity. Proc. Amer. Soc. Hort. Sci. **25**: 234–239. 1929.
17. LANTZ, C. W. Respiration in corn with special reference to catalase. Amer. Jour. Bot. **14**: 85–105. 1927.
18. LOEW, O. Catalase. U. S. Dept. Agr. Rept. **68**: 1–27. 1901.
19. MARKS, G. W. and FOX, D. L. The inactivation of mussel catalase by oxygen. Jour. Biol. Chem. **103**: 269–283. 1933.
20. MCLEOD, J. W. and GORDON, J. The problem of intolerance of oxygen by anaerobic bacteria. Jour. Path. & Bact. **26**: 332–343. 1923.
21. MORGULIS, S. and BEBER, N. Studies on the effect of temperature on the catalase reactions. VI. Heat inactivation of catalase at different hydrogen ion concentrations. Jour. Biol. Chem. **77**: 115–126. 1928.
22. MORINAGA, T. Catalase activity and the aerobic and anaerobic germination of rice. Bot. Gaz. **79**: 73–84. 1925.
23. NELLER, J. T. Relation of catalase activity to physiological breakdown in Jonathan apples. Plant Physiol. **6**: 183–189. 1931.
24. POPE, M. N. Catalase activity in relation to the growth curve in barley. Jour. Agr. Res. **44**: 343–355. 1932.
25. ————————. Catalase activity and respiration in the leaves of growing barley. Jour. Agr. Res. **45**: 35–40. 1933.
26. RHINE, L. E. Divergence of catalase and respiration in germination. Bot. Gaz. **78**: 46–67. 1924.
27. STERN, K. Über die katalatische Aktivität van Häminkomplexen. 5. Über Katalase. Zeitschr. physiol. Chem. **215**: 35–46. 1933.
28. ————————. Forgesetzte Versuch über synthetische Häminkatalasen. 7. Über Katalase. Zeitschr. physiol. Chem. **219**: 105–114. 1933.
29. SUMNER, J. B. and DOUNCE, A. L. Crystalline catalase. Science n. s. **85**: 366–367. 1937.
30. TAUBER, H. and KLEINER, I. S. Chemical nature of catalase. Proc. Soc. Exper. Biol. & Med. **33**: 391–392. 1935.
31. WOOLF, B. Recent advances in the study of enzymes. Nature (London) **129**: 928–930. 1932.
32. ZEILE, K. and HELLSTROM, H. Über die aktive Gruppe der Leberkatalase. Zeitschr. physiol. Chem. **191**: 171–192. 1930.

STUDIES ON THE PROTOPLASMIC NATURE OF STIMULATION AND ANESTHESIA. II[1]

HENRY T. NORTHEN

(WITH FIVE FIGURES)

Introduction

In a recent paper the writer (**12**) has discussed the protoplasmic nature of stimulation and anesthesia. It was believed that normal protoplasm has a grid structure which results from combinations of proteins with lipoids and other cell constituents. In unstimulated protoplasm the proteins are presumably in the form of long chains. Upon stimulation some of the proteins are separated from the grid and change from a chain into a globular form. The separation of the proteins from the grid and their change in shape results in a decrease in the structural viscosity of the protoplasm. All of the stimulating agents which we have studied cause a decrease in the structural viscosity of protoplasm (**12**). Since the cited paper was prepared the effects of other stimulating agents upon protoplasm in cells of Spirogyra have been studied and the data presented will demonstrate that electricity, disease, carbon dioxide, cold, and mechanical impacts cause a decrease in the structural viscosity of protoplasm.

If it be true that the primary effect of stimulating agents is a decrease in the structural viscosity of protoplasm which results from a partial breakdown of the protoplasmic network, then other effects of stimulating agents should result from the breakdown of the normal structure. Hence an attempt will be made to explain some of the secondary effects of stimulation on a protoplasmic basis.

In "dormant" cells stimulants often initiate growth. HEILBRUNN (**4**) states that some stimulating agents will initiate cell division in unfertilized animal eggs and many stimulating agents break rest periods in plants (**8**). How may the change in protoplasmic structure initiate growth? To a large extent the activities of a cell are determined by the protoplasmic pattern. In a dormant cell all requirements for growth may be present but the protoplasmic pattern is not favorable for growth. A stimulating agent breaks down the "dormant" protoplasmic pattern. The opportunity then exists for the network constituents to form a new pattern—one which may favor growth. It should also be mentioned that during mitosis and meiosis some of the cytoplasm becomes oriented and distributed. In animal cells amphiasters develop; in plant cells phragmoplasts. They develop in part from

[1] Contributions from the Department of Botany and the Rocky Mountain Herbarium of the University of Wyoming, no. 178.

the cytoplasm. The development of such structures necessitates the alteration of the protoplasmic pattern of resting cells so that the network constituents may become oriented to form the mentioned structures. Before rearrangement the original structure must be broken down by an external or internal stimulus; if other factors are present in the cell a pattern differing from the original will then be reconstructed and this new pattern may function differently. The breaking down and reconstruction involve changes in the consistency of protoplasm. Changes in the viscosity of protoplasm during mitosis and meiosis have been observed by HEILBRUNN (**3**), KOSTOFF (**5**), and others.

Most stimulating agents initially increase the rate of respiration (**6**). In unstimulated cells some of the substrate and respiratory enzymes are possibly "tied up" to the protoplasmic network. Upon stimulation, when the network is broken down, the enzymes and substrate may be released and accordingly the rate may be increased. It is believed that the increased respiratory rate furnishes the energy for recovery (usually changing the structure back to normal), an example of the auto-regulatory nature of protoplasm.

Stimulating agents generally increase the permeability rate (**4**). NORTHEN (**11**) deduced that the selectively permeable membranes have a structure comparable to that of the main mass of the cytoplasm but perhaps more compact. It may be stated tentatively that upon stimulation the structure is loosened, pores are increased in size, and concomitantly the rate with which certain substances may enter or leave is increased.

Response to stimulation is generally followed by conduction (**4**). A point stimulation will alter the structure locally; the local alteration will affect adjacent parts and hence will alter them. The newly altered structure will, in turn, affect the contiguous structure and in such manner the impulse may be conducted. This is in accordance with the data of NORTHEN and NORTHEN (**10**) who have found that the conduction of impulses through cells decreases the elasticity (structural viscosity) of the protoplasm.

Experimental results

EFFECTS OF ELECTRICITY ON THE STRUCTURAL VISCOSITY

NORTHEN and MACVICAR (**13**) have reported that direct currents initially decrease the elasticity (structural viscosity) of protoplasm in cells of the alga, Spirogyra. At that time, however, several important effects were overlooked because the electrodes were placed too close together and because the filaments were not carefully oriented on the microscope slides. In this part, the effects of a direct current on the structural viscosity of protoplasm in cells near the anode and near the cathode and the effects on cells distant from the electrodes will be described.

In this experiment a species of Spirogyra which had one chloroplast in each cell was used. Platinum electrodes with a diameter of 1.0 mm. were connected through an ammeter and rheostat to a source of current and were then placed 1.4 cm. apart on a strip of wet cotton which was partially immersed in water in a Petri dish. Portions of Spirogyra filaments which were 3.0 cm. long were then placed over the electrodes. In each experiment about 100 filaments were used. After a current of 2 ma. had flowed for the desired time (2, 4, 6, 8, 10, or 12 minutes) the filaments were centrifuged. To test whether the current had decreased the structural viscosity of the protoplasm the filaments were centrifuged with an acceleration of 382.5 × gravity for 90 seconds, a centrifugation which would not displace the chloroplasts in cells of control filaments. To test whether the structural viscosity had been increased a separate group of filaments was centrifuged for 40 seconds with an acceleration of 680 × gravity, an acceleration which would displace the chloroplasts in cells of control filaments. Following centrifugation the regions of the filaments where the chloroplasts were displaced and where they were not displaced were noted. The data are summarized in figure 1. The diagrams represent filaments which were exposed to the

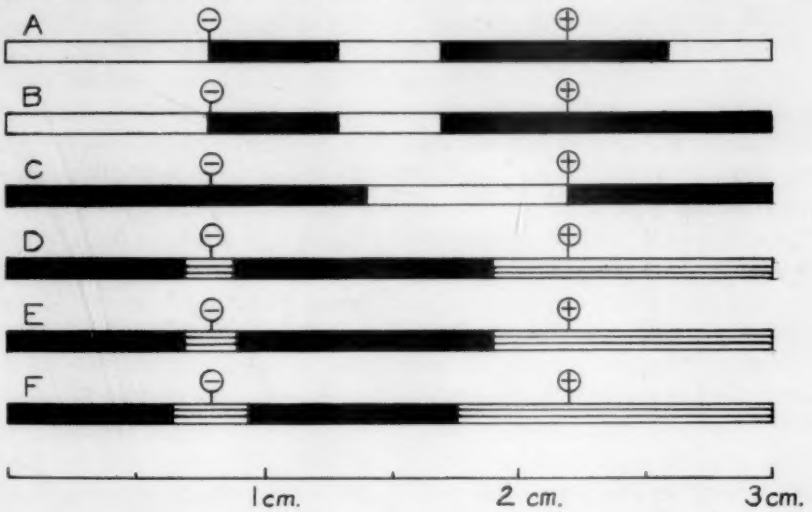

FIG. 1. Effects of a direct current on the structural viscosity of protoplasm.

current for 2 minutes (A), 4 minutes (B), 6 minutes (C), 8 minutes (D), 10 minutes (E), and 12 minutes (F). The white regions are regions where the structural viscosity of the protoplasm remained normal, the black regions are where the structural viscosity was decreased, and the lined regions are those in which the structural viscosity was increased. The structural viscosity was considered decreased if an acceleration of 382.5 × gravity dis-

placed the chloroplasts in cells which were exposed to the current. It will be recalled that such an acceleration would not displace the chloroplasts in control cells. In such cells the chloroplasts were not displaced by an acceleration of 382.5 × gravity because protoplasm has a yield value and the force produced by that acceleration was equal to or less than the yield value. Protoplasm is thus an elastic fluid, a fluid which possesses structural viscosity. NORTHEN (9) has demonstrated that the velocity of chloroplastic movement in cells of Spirogyra can be calculated approximately from the equation, $V = k(c - c_o)$. In the equation, V is the velocity of chloroplastic movement, k is a constant, c is the centrifugal acceleration used, and c_o is the initial starting acceleration (the yield acceleration). Hence for control filaments when an acceleration of 382.5 × gravity was used $V = k(382.5 - 382.5) = 0$ or $V = k(382.5 - > 382.5) = < 0$.

In filaments which were exposed to the current the chloroplasts were moved in some regions by an acceleration of 382.5 × gravity since the current had loosened the protoplasmic network and concomitantly decreased the value of c_o. Hence in such regions $V = k(382.5 - < 382.5) = > 0$. A decrease in the value of c_o has been interpreted as a decrease in the structural viscosity of the protoplasm.

On the other hand, in regions (those lined) exposed to the current and where the chloroplasts could not be moved by an acceleration of 680 × gravity, the value of c_o had been increased to at least 680 × gravity. (In control filaments the value of c_o was less than 680 × gravity but equal to or greater than 382.5 × gravity). An increase in the value of c_o indicates that the structural viscosity of the protoplasm has been increased.

It will be noted, figure 1A, that after two minutes exposure to the current the structural viscosity has been decreased only in the vicinity of the electrodes. Likewise when isolated muscle fibers are stimulated electrically the response may be localized at the electrodes (1).

With exposures of eight or more minutes the structural viscosity is decreased on the cathodal side except in the immediate vicinity of the cathode but increased on the anodal side. It is likely that the protoplasm with increased structural viscosity (which probably indicates that the protoplasmic network has been tightened) is not as sensitive to stimulants as is normal protoplasm and hence may be considered anesthetized. This observation is in accord with the statement by HEILBRUNN (4) that the passage of an electric current may produce a relative insensitivity at the anode.

EFFECT OF DISEASE ON THE STRUCTURAL VISCOSITY

The data presented suggest that the structural viscosity of protoplasm in cells infected with a Chytrid is less than the structural viscosity of protoplasm in normal cells of Spirogyra.

Two species of Spirogyra were found which were infected by Chytrids. The filaments were centrifuged with an acceleration of 382.5 × gravity for one minute, a centrifugation which would not displace the chloroplasts in normal cells. Following centrifugation observations were made to determine whether or not such an acceleration would displace the chloroplasts in infected cells.

The data are summarized in figure 2. Numbers 1 and 2 represent uncen-

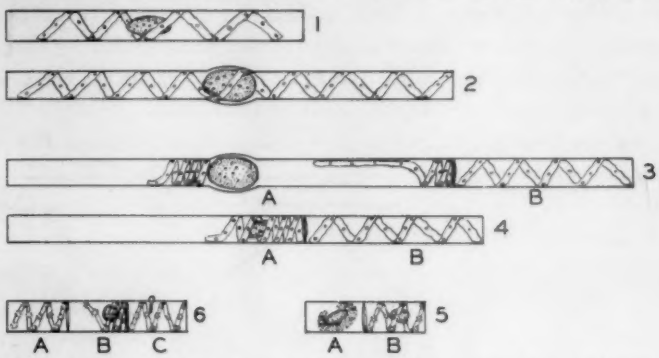

FIG. 2. Effects of disease on the structural viscosity of protoplasm.

trifuged infected cells of Spirogyra 1. Numbers 3 and 4 represent cells from centrifuged filaments. It will be noted nos. 3 and 4 (cells A) that the chloroplasts are displaced in the infected cells and are not displaced in the uninfected cells (cells B). The chloroplasts were probably displaced in the infected cells because the infection caused a decrease in the structural viscosity of the protoplasm. Number 5 represents uncentrifuged cells of Spirogyra 2. In cell A of number 5 the protoplasm has disintegrated and in such cells centrifugation caused no displacement of the cell contents. Number 6 represents centrifuged cells of Spirogyra 2. In the uninfected cell (A) the chloroplast has not been displaced. The chloroplast, however, has been displaced in the infected cell (B), and this indicates that the structural viscosity has been decreased. In cell C infection is just starting and as yet the structural viscosity has not been affected.

EFFECT OF CARBON DIOXIDE ON STRUCTURAL VISCOSITY

Carbon dioxide often acts as a stimulating agent. THORNTON (**15**) reports that carbon dioxide is effective in breaking the dormancy of potato tubers. LILLIE (**7**) states that the respiratory center of vertebrates increases its rhythm as carbon dioxide accumulates in the blood. Carbon dioxide may also stimulate the growth of bacteria (**16**). The data to be presented will demonstrate that the carbonate ion causes a decrease in the structural viscosity of protoplasm in cells of Spirogyra. The decrease in structural

viscosity may result from the separation of network constituents and the subsequent change in shape of protein molecules.

One group of Spirogyra filaments was placed in tap water, another in tap water which was maintained charged with carbon dioxide, another in tap water maintained at a pH of 5.6 (the pH of the charged water) through the addition of hydrochloric acid, and still another group were placed in tap water maintained at pH 5.6 through the addition of acetic acid. After the filaments had been in the solutions the desired time they were centrifuged (for immersion times and centrifugations consult table I). Following centrifugation the percentages of filaments in the cells of which the chloroplasts had been displaced were determined.

The data are summarized in table I and part of the data are expressed

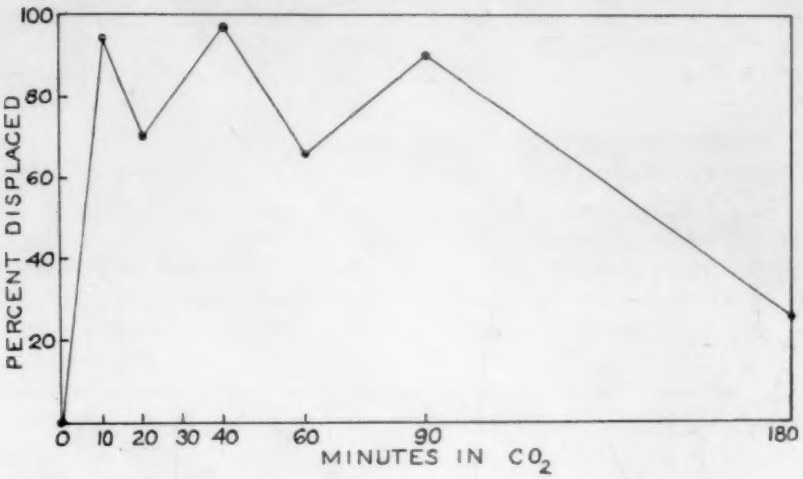

FIG. 3. Effects of tap water charged with carbon dioxide on the structural viscosity of protoplasm. The higher the point on the curve the lower the structural viscosity of the protoplasm.

graphically in figure 3. It will be noted that in those filaments which were exposed to carbon dioxide for 90 minutes or less the chloroplasts were displaced in most cases by an acceleration of 170 × gravity whereas in the control filaments even an acceleration of 680 × gravity was not sufficient to displace the chloroplasts in most of the cells. With exposures to carbon dioxide of 180 and 360 minutes the chloroplasts were displaced in cells of most filaments by an acceleration of 382.5 × gravity. Hence the data indicate that the carbonate ions decidedly decrease the structural viscosity of the protoplasm. The carbonate ions rather than the H ions cause the decrease since acidification of the tap water with hydrochloric or acetic acid did not decidedly affect the structural viscosity. The above experiment was re-

TABLE I

EFFECTS OF CARBON DIOXIDE ON THE STRUCTURAL VISCOSITY OF PROTOPLASM

TIME IMMERSED	CENTRIFUGATION	FILAMENTS IN THE CELLS OF WHICH THE CHLOROPLASTS WERE DISPLACED WHEN IMMERSED IN			
		TAP WATER	TAP WATER AND HCl	TAP WATER AND ACETIC ACID	TAP WATER AND CO_2
min.		%	%	%	%
10	170 ×g. for 2 min.	0	4	4	94
20	"	0	0	1	70
40	"	0	0	2	97
60	"	0	0	6	66
90	"	0	0	17	90
180	"	0	0	0	25
180	382.5 ×g. for 2 min.	4	3	32	84
360	"	1	2	24	92
360	680 ×g. for 2 min.	6	.....	.....	.....

peated with a different species of Spirogyra with essentially the same results.

In table II data are recorded which indicate that in most filaments recovery from the effects of carbon dioxide occurs in less than five minutes. In this experiment filaments were immersed in water charged with carbon dioxide for five minutes and were then transferred to ordinary tap water. After they had been in the tap water for various periods of time the filaments were centrifuged with an acceleration of 170 × gravity for two minutes. Such a centrifugation did not displace the chloroplasts in cells of control filaments.

TABLE II

TIME REQUIRED FOR THE PROTOPLASM TO RECOVER FROM CARBON DIOXIDE

TIME IN NON-CHARGED WATER	FILAMENTS IN THE CELLS OF WHICH THE CHLOROPLASTS WERE DISPLACED
min.	%
1	95
5	17
10	16
15	1
20	3
30	1

EFFECTS OF COLD ON STRUCTURAL VISCOSITY

If organisms are maintained at low temperatures they may become anesthetized (**14**). ETS (**2**) further reports that two types of blocks occur when nerves are locally cooled. In some nerves a block does not occur until after ice has formed (freezing type), whereas in others a block occurs without ice formation. In the latter (non-freezing type) the nerves recover

when the temperature is raised. The cold anesthesia and the non-freezing type of block may be explained on the basis of an increase in the structural viscosity of the protoplasm. NORTHEN (**11**) has found that the structural viscosity of the protoplasm in cells of Spirogyra increases when filaments are maintained at 1.0° C. which indicates that the protoplasmic network is tightened and accordingly may not break down as readily as the normal network upon stimulation. In other words, the tightened network may be anesthetized. The freezing type of block, one which does not regain conduction when transferred to room temperatures, may be the result of a localized coagulation of the protoplasm. The data presented indicate that temperatures below freezing cause coagulation of protoplasm in Spirogyra.

Not only may low temperature act as an anesthetic but it may also act as a stimulating agent. For example, MAXIMOV (**8**) states that freezing may be effective in breaking rest periods in plants. HEILBRUNN (**4**) states that cold may often act as a stimulating agent. Stimulating agents primarily decrease the structural viscosity of protoplasm and anesthetics either increase the structural viscosity or change the pattern from a sensitive to a relatively insensitive one (**12**). How then may low temperatures act both as anesthetizing and stimulating agents? When an organism is maintained at a low temperature it may become anesthetized but when it is transferred from a low to a higher temperature the stimulating effect may become apparent. In other words, the stimulation may not occur at the low temperature but only after the organism is transferred to a higher temperature. The data to be presented will demonstrate that the structural viscosity of protoplasm in filaments of Spirogyra which were subjected to low temperatures and then transferred to room temperature is less than the structural viscosity of protoplasm in filaments which were not subjected to low temperatures but which were only maintained at room temperatures. Hence cold may be classified as a stimulating agent because it may indirectly cause a decrease in the structural viscosity of protoplasm.

Petri dishes were filled to a depth of about 2 mm. with water at a temperature of 15° C. Filaments of Spirogyra were then placed in the dishes. They were next placed in a refrigerator which was maintained at approximately −3.0° C. After the dishes had been in the refrigerator the desired time they were removed. The filaments were then transferred to water which had a temperature of 20.0° C. The filaments were allowed to remain in this water for various periods of time and they were then centrifuged. Following centrifugation the percentages of filaments in the cells of which the chloroplasts had been displaced (moved to centrifugal end) were determined for experimental and control groups.

The data are summarized in table III and expressed graphically in figure 4.

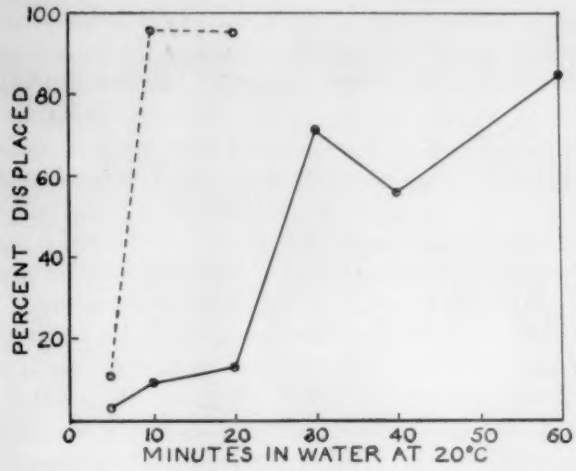

FIG. 4. Effects of previous exposure to cold on the structural viscosity of protoplasm. The filaments were allowed to remain at – 3.0° C. for 10 minutes (unbroken line) and 20 minutes (broken line). They were then transferred to water at 20° C. where they were allowed to remain for the indicated times and finally the filaments were centrifuged. The higher the points on the curves the lower the structural viscosity.

It will be noted that following exposures to cold there is a latent period, a period during which the protoplasm of those exposed to cold is apparently like that of those which were not exposed to cold. For those filaments which remained in the refrigerator 20 minutes the latent period was less than 10

TABLE III

EFFECT OF LOW TEMPERATURES ON THE STRUCTURAL VISCOSITY OF PROTOPLASM

TIME FILAMENTS WERE IN REFRIGERATOR	REMARKS	CENTRIFUGATION	PERIOD BETWEEN REMOVAL FROM COLD AND CENTRIFUGATION	FILAMENTS IN WHICH CHLOROPLASTS WERE DISPLACED
min.			*min.*	%
0.0	Control	680 × g. for 0.5 min.	.....	2
0.0	"	1062 × g. for 0.5 min.	.....	18
10.0	Ice just forming over surface of water	680 × g. for 0.5 min.	5.0	3
"	"	"	10.0	9
"	"	"	20.0	13
"	"	"	30.0	71
"	"	"	40.0	56
"	"	"	60.0	84
20.0	Much of water frozen but not directly around filaments	1062 × g. for 0.5 min.	5.0	11
"	"	"	10.0	96
"	"	"	20.0	95

minutes. It will be noted that 10 minutes after the filaments were removed from the cold water the chloroplasts were displaced in the cells of 96 per cent. of the filaments whereas in control filaments a like centrifugation displaced the chloroplasts in only 18 per cent. of the filaments. Hence it is concluded that previous exposure to cold may result in a decrease in the structural viscosity of protoplasm and accordingly cold may be considered a stimulating agent.

During the course of the main experiment several other experiments were performed and these will be discussed. For these experiments a different species of Spirogyra was used. In this species an acceleration of 382.5 × gravity displaced the chloroplasts in the cells of most untreated filaments. One group of filaments was allowed to remain in 0.2 molal sucrose for six hours. They were then transferred to water and placed in a refrigerator maintained at −3.0° C. Another group of filaments, which had not previously been in sucrose, were likewise placed in the refrigerator. After one hour all of the water in the Petri dishes was frozen. The dishes were then removed and the ice was allowed to thaw. The filaments were then transferred to water at 20° C. and remained at that temperature for ½ hour, 1 hour, 2 hours, and 4 hours, respectively. Finally the filaments were centrifuged with an acceleration of 382.5 × gravity. The acceleration would not displace the chloroplasts in those cells which had previously been in sucrose nor in those cells which had not previously been in sucrose. In both instances the protoplasts had shrunken from the cell walls (figures 5A and 5B). The observations indicate that the freezing had coagulated the protoplasm and that previous immersion in sucrose did not retard the coagulation.

It is likely, however, that the coagulation was not the result of the withdrawal of water and the concomitant increase in the concentration of electrolytes since withdrawal of water by hypertonic sucrose solutions does not cause coagulation (**12**). At the time the freezing experiments were performed filaments of Spirogyra were plasmolyzed in 0.8 molal sucrose. After the filaments had been in the solution for the desired time they were centrifuged with an acceleration of 382.5 × gravity. After the filaments had been in the sucrose solution for ½ hour, 1 hour, 2 hours, or 4 hours the protoplasm was not coagulated as evidenced by the fact that the centrifugation displaced the chloroplasts in most cells. Cells which were allowed to remain in the sucrose solution for 2 hours are diagrammed in figures 5C and 5D. It will be noted that the chloroplasts have been moved by the centrifugal acceleration.

Effects of mechanical impacts on the structural viscosity of protoplasm

The data presented will show that mechanical impacts decrease the struc-

tural viscosity of protoplasm in cells of Spirogyra, that recovery usually occurs in less than 10 minutes, and that stimuli are additive.

Filaments of Spirogyra were placed in water on a slide and were covered with a cover glass. A glass cylinder which surrounded a steel upright was then allowed to fall on the filaments. Following the impulse the filaments were centrifuged and the percentages of filaments in the cells of which the chloroplasts were displaced were determined.

The effects of a 3.20 gram weight dropping distances of 10, 15, 20, 25, and 30 cm. on the structural viscosity of protoplasm are recorded in table IV. In all instances the filaments were centrifuged with an acceleration of 245 × gravity for 2 minutes.

TABLE IV

EFFECTS OF MECHANICAL IMPACTS ON THE STRUCTURAL VISCOSITY OF PROTOPLASM

IMPULSE	FILAMENTS IN THE CELLS OF WHICH THE CHLOROPLASTS WERE DISPLACED
ergs.	%
000.0	1
448.0	8
548.5	35
633.6	32
708.4	51
775.3	72

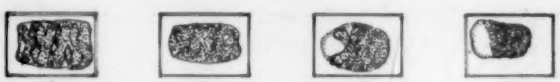

FIG. 5. A and B represent centrifuged cells which remained in 0.2 molal sucrose for six hours before being placed in a refrigerator at −3.0° C. It will be noted that the chloroplasts have not been moved by the centrifugal acceleration. C and D represent cells which remained in 0.8 molal sucrose for two hours prior to centrifugation. It will be noted that the centrifugation has displaced the chloroplasts in the protoplasts.

It will be noted that in filaments which were subjected to mechanical impacts the chloroplasts were displaced in greater percentages of filaments than in the controls. This indicates that mechanical impacts decrease the structural viscosity of protoplasm probably through loosening the protoplasmic network.

TIME FOR RECOVERY FROM IMPACTS.—Filaments of Spirogyra were subjected to an impulse of 775.3 ergs. After the impulse the filaments were allowed to remain in water for various periods of time before centrifugation. The results are recorded in table V.

At the time experiment no. 1 was performed more than eight minutes were required for complete recovery whereas at the time no. 2 was performed

TABLE V

TIME FOR RECOVERY FROM MECHANICAL IMPACTS

EXPERIMENT NUMBER	CENTRIFUGATION	INTERVAL BETWEEN IMPACT AND CENTRIFUGATION	FILAMENTS IN CELLS OF WHICH THE CHLOROPLASTS WERE DISPLACED IN	
			EXPERIMENTAL GROUPS	CONTROL GROUPS
		sec.	%	%
1	245 × g. for 2 min.	15	90	
	"	120	75	42
	"	240	75	
	"	480	54	
2	170 × g. for 2 min.	15	40	
	"	60	26	3
	"	120	4	

recovery occurred in about two minutes as evidenced by the fact that in the controls the chloroplasts were displaced in 3 per cent. of the filaments and in the experimental filaments in 4 per cent. The difference is not considered significant.

SUMMATION OF MECHANICAL STIMULI.—Filaments of Spirogyra were given one mechanical impact and then after various periods of time had elapsed they were given a second impact. To obtain intervals of less than one-half second two weights were dropped simultaneously from different heights. Following the second impact the filaments were centrifuged for two minutes with an acceleration of 170 × gravity.

The data are summarized in table VI.

The data in table VI indicate that mechanical impacts are additive. In experiment 1 the chloroplasts were displaced in 19 per cent. of the filaments which were subjected to a single impulse of 775.3 cgs. and in 17 per cent. of the filaments which were subjected to an impulse of 678.4 cgs. However, if an impulse of 775.3 cgs. were followed a second later by an impulse of 678.4 cgs. the chloroplasts were displaced by the centrifugation in 80 per cent. of the filaments. Likewise in experiments 2 and 3 it will be observed that impulses are additive. In experiments 2 and 3 a single impulse of 1455 cgs. did not have as great an effect as two separate impulses. For example, in experiment 2 centrifugation displaced the chloroplasts in 48 per cent. of the filaments which were given one impulse of 1455 cgs. but displaced the chloroplasts in 71 per cent. of the filaments which were given an impulse of 775.3 cgs. and then 30 seconds later a second impulse of 678.4 cgs. (total = 1437 cgs.). Apparently following the first impact the protoplasmic network is more sensitive to stimulation than it was originally. In experiment 1 the protoplasm was decidedly hypersensitive 0.045 second

TABLE VI

ADDITION OF MECHANICAL STIMULI

EXPERIMENT NUMBER	IMPULSE OF FIRST IMPACT	IMPULSE OF SECOND IMPACT	TIME BETWEEN IMPULSES	FILAMENTS IN CELLS OF WHICH THE CHLOROPLASTS WERE DISPLACED	
	cgs.	*cgs.*	*sec.*	%	
1	0.0	0.0	.....	1	(water control)
	775.3	0.0	.....	19	
	678.4	0.0	.....	17	
	775.3	701.2	0.008	42	
	708.4	678.4	0.022	43	
	633.6	678.4	0.045	71	
	775.3	678.4	1.0	80	
	775.3	678.4	15.0	59	
	775.3	678.4	120.0	64	
	775.3	678.4	240.0	30	
	775.3	678.4	480.0	8	
2	0.0	0.0	.....	3	(water control)
	775.3	0.0	.....	41	
	678.4	0.0	.....	40	
	1455.0	0.0	.....	48	
	775.3	701.2	0.008	33	
	708.4	678.4	0.022	31	
	633.6	678.4	0.045	42	
	775.3	678.4	1.0	40	
	775.3	678.4	15.0	64	
	775.3	678.4	30.0	71	
	775.3	678.4	60.0	64	
	775.3	678.4	120.0	42	
	775.3	678.4	240.0	11	
3	0.0	0.0	.....	0	(water control)
	678.4	0.0	.....	4	
	1455.0	0.0	.....	46	
	775.3	678.4	0.5	81	
	775.3	678.4	1.0	66	
	775.3	678.4	2.0	90	
	775.3	678.4	4.0	12	
	775.3	678.4	8.0	40	
	775.3	678.4	16.0	15	
	775.3	678.4	32.0	24	
	775.3	678.4	60.0	32	
	775.3	678.4	120.0	18	
	775.3	678.4	240.0	16	
	775.3	678.4	480.0	9	
	775.3	678.4	960.0	7	

after the first impulse, in the second 15 seconds after the first impulse, and in the third 0.5 second after the first impulse.

Following the period of hyper-sensitivity there may or may not be a period of hypo-sensitivity. The data for experiments 1 and 2 suggest that 480.0 and 240.0 seconds, respectively, after the first impulse the protoplasm is less sensitive than it was originally. However, in experiment three the protoplasm was more sensitive to stimulation 240 and 480 seconds after the first impact than it was originally.

SUMMATION OF ETHER STIMULATION AND MECHANICAL IMPACTS.—In this experiment filaments of Spirogyra were immersed in 0.3 per cent. ether, which is far less than the anesthetic concentration, for various periods of time. Some of the filaments were then subjected to an impulse of 678.4 cgs. Following the impulse the filaments were centrifuged. Filaments which had received only the ether treatment, filaments which had been given only an impulse, and normal filaments were likewise centrifuged. The results are tabulated in table VII.

TABLE VII

SUMMATION OF ETHER STIMULATION AND MECHANICAL IMPACTS

CENTRIFUGATION	IMMERSION IN ETHER	FILAMENTS IN THE CELLS OF WHICH THE CHLOROPLASTS WERE DISPLACED WHEN			
		IMMERSED IN 0.3 PER CENT. ETHER	IMMERSED IN 0.3 PER CENT. ETHER AND THEN GIVEN IMPULSE	WATER ONLY	IMPULSE ONLY
	min.	%	%	%	%
170 × g. for 2 min.	6	6	52		
"	10	8	95	2	17
"	20	3	3		
"	40	3	6		
245 × g. for 2 min.	6	47	—		
"	10	40	—	23	62
"	20	53	—		
"	40	28	64		

Initially 0.3 per cent. ether causes a slight decrease in the structural viscosity of protoplasm. This is clearly indicated in the filaments which were centrifuged with an acceleration of 245 × gravity. Here it will be noted that the chloroplasts are displaced in greater percentages of filaments than in the water controls. With both accelerations mechanical impacts cause a decrease in the structural viscosity of the protoplasm as evidenced by higher percentages of displacements than in the water controls. The filaments which were centrifuged with an acceleration of 170 × gravity demonstrate that there is a summation of stimuli when the filaments were immersed for 6 or 10 minutes in the dilute ether. For example, the chloroplasts were displaced in 8 per cent. of the filaments which were subjected to 0.3 per cent. ether for 10 minutes and were displaced in 17 per cent. of the filaments which were subjected to the impulse but were displaced in 95 per cent. of the filaments which were immersed for 10 minutes in ether prior to the impulse.

Summary

A current of 2 ma. acting for 2 minutes caused a decrease in the structural viscosity of Spirogyra protoplasm in the vicinity of the electrodes.

With durations up to six minutes the decrease in structural viscosity spread to other cells. After more than 6 minutes exposure to the current the structural viscosity increased on the anodal side of the filaments and in a small region near the cathode and decreased in regions adjacent to the region of increased structural viscosity near the cathode. In this and subsequent experiments the structural viscosity was considered decreased if an acceleration which would not displace the chloroplasts in cells of normal filaments displaced the chloroplasts in cells of experimental filaments. The structural viscosity was considered increased if a centrifugal acceleration which displaced the chloroplasts in cells of control filaments would not displace the chloroplasts in experimental cells.

Disease, carbon dioxide, previous exposure to cold, and mechanical impacts caused a decrease in the structural viscosity of the protoplasm in cells of Spirogyra. In most filaments recovery from the effects of carbon dioxide and mechanical impacts occurred in less than 10 minutes.

The effects of two successive mechanical stimuli were additive. Stimulation by dilute ether and mechanical impacts were also additive.

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LITERATURE CITED

1. BROWN, D. E. S. and SICHEL, F. J. M. The isometric contraction of isolated muscle fibers. Jour. Cell. and Comp. Physiol., **8**: 315–328. 1936.
2. ETS, H. N. The effect of potassium on the cold blocking of spider crab nerve. Jour. Cell. and Comp. Physiol., **8**: 101–108. 1936.
3. HEILBRUNN, L. V. The colloid chemistry of protoplasm. Berlin. 1928.
4. ————. An outline of general physiology. Philadelphia. 1937.
5. KOSTOFF, D. Protoplasmic viscosity of dividing cells in floral buds of tobacco. Protoplasma **11**: 177–183. 1930.
6. KOSTYCHEV, S. Plant respiration. Philadelphia. 1927.
7. LILLIE, R. S. Protoplasmic action and nervous action. Chicago. 1932.
8. MAXIMOV, N. A. Plant physiology. New York. 1938.
9. NORTHEN, H. T. Studies of protoplasmic structure in Spirogyra. I. Elasticity. Protoplasma **31**: 1–8. 1938.
10. ———— and NORTHEN, R. T. Studies of protoplasmic structure in Spirogyra. II. Alterations of protoplasmic elasticity. Protoplasma **31**: 9–19. 1938.
11. ————. Studies of protoplasmic structure in Spirogyra. IV. Effects of temperature on protoplasmic elasticity. Bot. Gaz. **100**: 619–626. 1939.
12. ————. Studies on the protoplasmic nature of stimulation and anesthesia. Cytologia **10**: 105–112. 1939.

13. ———— and MacVicar, R. Studies of protoplasmic structure in Spirogyra. VI. Effects of sound and electricity on protoplasmic elasticity. Cytologia **10**: 18–22. 1939.

14. Parker, G. H. General anesthesia by chilling. Science n. s. **90**: 63. 1939.

15. Thornton, N. C. Carbon dioxide storage. XIII. Relationship of oxygen to CO_2 in breaking dormancy of potato tubers. Contr. Boyce Thompson Inst. **10**: 201–204. 1939.

16. Valley, G. and Rettger, L. F. The influence of carbon dioxide on bacteria. Jour. Bact. **14**: 101–137. 1927.

PHYSIOLOGICAL FACTORS OPERATIVE IN ICE-SHEET INJURY OF ALFALFA[1]

V. G. Sprague and L. F. Graber

(WITH FIVE FIGURES)

Introduction

Ice-sheets that contact the surface parts of herbaceous plants for extended periods are among the most injurious of the climatic hazards of many overwintering crops. Under field conditions they occur primarily in low spots which are not well drained. They may, however, cover large areas regardless of topography because sleet storms prevail in latitudes between those regions which are snow-covered for the major portion of the winter and those which, for the most part, are bare.

Knowledge of the nature of ice-sheet injury is limited, and the work reported here is quite preliminary. It is an attempt to ascertain under controlled conditions some of the basic physiological factors which cause this type of winter injury. It has been suggested that killing by ice-sheets is due to smothering. The exact meaning or interpretation which that word was intended to convey is not clear. Bugaevskii and Zitnikova (**1**) have observed that winter wheat plants beneath an ice crust at $-1°$ C. to $-9°$ C. began to decrease in vigor after the twenty-third day and in fifty-four days all were dead. They suggest a lack of oxygen as a possible cause of death. On the other hand, smothering might be construed to mean the absence of a free diffusion of substances from the organism into the pore spaces of the soil and into the air or conversely, from the air to the organism. Whatever the interpretation of the term may be, efforts to break the ice cover and to eliminate its sealing effects by mechanical means such as disking and other types of scarification, have not proven effective in reducing or preventing ice-sheet injury under field conditions. In 1922, Graber scarified alternate areas of an ice sheet on an alfalfa field in Wisconsin with a weighted disk. Although the scarification occurred within four days after the formation of the ice-sheet the alfalfa was badly killed whether the ice was broken or allowed to remain intact. He further observed that during the first three months of 1937, a sheet of ice, frozen tightly to the soil surface, covered most of the southeastern quarter of Wisconsin. In desperation, many farmers scarified this ice cover with disks and other farm implements. In no case was such treatment reported as being helpful and the losses from winter-killing of alfalfa that year were in excess of 200,000 acres. Only where an interven-

[1] Contribution no. 151 from the Department of Agronomy, Wisconsin Agricultural Experiment Station, Madison, Wis. Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

ing layer of snow prevailed between the ice and the soil, did the alfalfa escape injury.

Experiment I

Methods and procedure

Experiments were begun in the fall and early winter of 1934 to study some of the physiological factors causing ice-sheet injury of alfalfa (*Medicago sativa*). The winter-hardy Grimm alfalfa used in this experiment was sown on the University Farm at Madison, May 15, 1934. Although the plants grew vigorously, the top growth was not cut and the plants entered the dormant winter condition with a high storage of organic reserves. By December 22, 1934, the soil was frozen to a depth of two inches and the plants were well hardened. On this date, the plants were dug from the field and were hurriedly washed with cold water. The tops were removed 2 or 3 inches above the crown, and the roots (with crowns) were stored in damp towels at $-8°$ to $-10°$ C. until needed.

On January 5, 1935, the dormant plants were taken from the cold room to the laboratory and arranged into samples of 10 roots each, selecting as nearly uniform plants as possible. These were trimmed to 10 cm. from the cotyledonary node downward while the crown was trimmed to 2 cm. from the cotyledonary node upward. Twenty-six samples of 10 plants each were placed in twenty-six large Pyrex test tubes. Twelve were stoppered with rubber stoppers and had air as the surrounding medium; twelve more were filled with nitrogen, by displacement of water, and stoppered; the remaining two tubes were provided with an inlet and outlet tube through which air was introduced continuously at a constant rate.

On the above date, twelve additional samples of ten plants each were frozen in solid cakes of ice. This was effected by making heavy collodion bags in a $1\frac{1}{2}'' \times 8''$ test tube and, while filled with warm water, varnishing

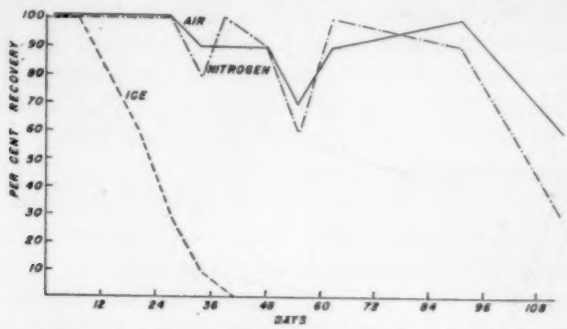

Fig. 1. Percentages of survival after hardened Grimm alfalfa plants were stored in ice and stagnant gaseous media at $-3.5°$ C. for 113 days. Survival values were ascertained by exposure of the plants to favorable conditions for growth in the greenhouse for 3 weeks. Experiment I. Series of January 7 to May 3, 1935.

them with two coats of a high grade water-spar varnish which was impermeable to the alcohol in the freezing bath. A sample of ten plants was placed in each of twelve such bags. The roots were evenly spaced within the bag, covered with tap water, and a cork securely fastened into the top. These samples and those in stoppered test tubes were then suspended in an alcohol-water bath at −3.5° C. They were maintained at this temperature until removed at intervals of several days, when they were thawed at 1° to 2° C. After thawing they were transplanted into sand and placed in the greenhouse for three weeks when determinations of survival and measurements of top growth were made. These are presented in table I and figures 1 and 2.

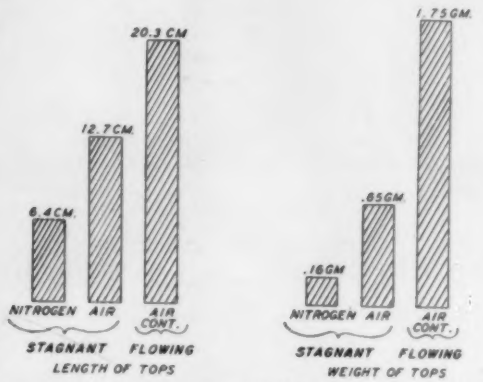

FIG. 2. Vigor of alfalfa plants expressed by the average length of stems and dry weight of top growth after 3 weeks of exposure to favorable growth conditions in the greenhouse. Such plants had been subjected to storage in test tubes of gaseous media for 113 days at −3.5° C. Experiment I. Series of January 7 to May 3, 1935.

VIGOR AND SURVIVAL AS INFLUENCED BY STORAGE IN VARIOUS MEDIA

With only 7 days of storage at −3.5° C., all the plants survived regardless of the surrounding media. With greater duration of the treatments, the plants in ice were first to decline in vigor and percentage of survival, followed somewhat later by the samples stored in stagnant nitrogen, and, finally, by the samples stored in stagnant air. The plants maintained in ice for more than 7 days declined very rapidly and at the end of 41 days all were dead. The plants maintained in stagnant nitrogen for more than 27 days were weakened and recovery was slow, but 90 per cent. were still alive at 91 days and 30 per cent. at 113 days. The plants kept in stoppered tubes of air had higher survival and stronger recovery than those maintained in nitrogen while those in flowing air (table I) survived 100 per cent. and showed only slightly reduced vigor even after 113 days of storage. The latter were much more vigorous than those stored in the stoppered tubes of air or nitrogen. These results are illustrated in figure 2.

TABLE I

GROWTH RECOVERY OF HARDENED ALFALFA PLANTS AFTER HAVING BEEN STORED IN VARIOUS MEDIA FOR VARYING PERIODS AT −3.5° C. EXPERIMENT I. SERIES OF JANUARY 7, 1935, TO MAY 3, 1935

DAYS STORED	PERCENTAGE SURVIVAL WHEN STORED IN			
	ICE	N_2 (STAGNANT)*	AIR (STAGNANT)*	AIR (FLOWING)
	%	%	%	%
2	100	100	100	.....
7	100	100	100	.....
20	60 (weak)	100	100	.....
27	30 (weak)	100	100	.....
34	10 (weak)	80 (weak)	90	.....
41	0	100 (weak)	90	.....
48	0	90 (weak)	90	.....
55	0	60 (weak)	70	.....
62	0	100 (weak)	90	.....
91	0	90 (weak)	100	.....
113	0	30 (weak)	60 (weak)	100 (strong)

* Test tubes containing plants were stoppered.

Experiment II

METHODS AND PROCEDURE

The plants used in this experiment were selected from a lot of very winter-hardy Turkestan alfalfa. The seed was sown on the University Farm, July 25, 1935, in a rich loam soil and with favorable weather the plants attained a good size by the time fall hardening began. On November 30, 1935, several thousand plants were dug from the field. The plants with very small and extremely large roots were discarded and 1200 fairly uniform plants were thus obtained. Of these, 200 were reset in each of six tin boxes and buried in the field to ground level. This provided an abundant and efficient source of hardened material without entailing the difficulties of removing individual plants from frozen soil. By using this method of hardening and storing in the field, the plants could easily be taken at intervals during the winter to a cool greenhouse for gradual thawing and removal from the containers. After thawing, the plants were taken to the laboratory, hurriedly washed in cold tap water, trimmed to root lengths of 10 cm. and to crown lengths of 2 cm. as before. These were grouped into uniform samples of ten plants each.

In this experiment the hardened plants were exposed to a temperature of −4° C. A continuous flow of air was drawn through the test tubes containing one series of plants; a flow of nitrogen through another, and a flow of carbon dioxide through another. One series was frozen in ice as in Experiment I.

In Experiment I some difficulty was encountered with the plants frozen in ice because the collodion bags coated with varnish became brittle and ex-

tremely fragile when subjected to the freezing temperatures. In some cases this resulted in cracked bags which allowed the alcohol of the bath to enter. These samples were, of course, discarded. To overcome this difficulty, rubber latex tubes approximately $1\frac{1}{2}'' \times 7''$ were obtained and the roots were placed in these. The individual plants were separated within the ice as in the previous experiment. The tubes were filled with cold tap water and the tops of the tubes were closed with a large cork before suspension in an alcohol-water bath at $-4°$ C.

The samples to be frozen in circulating air, nitrogen, and carbon dioxide were placed in $1'' \times 8''$ Pyrex test tubes which were fitted with an inlet tube extending just below the stopper and an outlet tube extending to the bottom of the test tube. This insured a constant flow of the gases through the test tubes without stratification. The test tubes through which each gas was introduced were arranged in series and connected by means of short pieces of rubber tubing. Between the root samples and the gas supplies Erlenmeyer flasks, filled with finely chopped ice to saturate the gas with water vapor, were inserted.

The compressed air line in the laboratory provided the source of air. A pressure regulator was installed to provide a slow but constant flow. The nitrogen was obtained by passing air through two large absorption bottles containing alkaline pyrogallate. It was then washed in water and concentrated sulphuric acid. The carbon dioxide was obtained from dry ice. A 2-pound piece of solid carbon dioxide was wrapped in cheese cloth and placed in a gallon thermos jug fitted with a large one-hole rubber stopper. The jug was placed in the freezing chamber to prevent too rapid evolution of the carbon dioxide. The gas was washed in concentrated sulphuric acid and the pressure and rate of flow regulated automatically.

The tubes containing the plants were properly connected with the sources of gas and placed in the thermostatically regulated alcohol-water bath at $-4°$ C. Samples of all series were removed at intervals of about 7 days. The roots were allowed to thaw at 1° to 2° C. for 10 to 12 hours, after which they were transplanted into quartz sand for recovery in the greenhouse. The extent of injury was measured by the weight of dry matter of tops obtained from the various samples after three weeks of growth. Greenhouse conditions were not entirely uniform for each three weeks' period—varying considerably in the amount of sunlight during the winter months. However, the relative growth of the samples tested in any single three weeks' period was comparable.

Effects of storage in various media in experiment II

The results of this experiment are recorded in table II and are presented in graphic form in figure 3. It is evident that little injury takes place with

TABLE II

RECOVERY OF HARDENED ALFALFA PLANTS AFTER STORAGE IN VARIOUS MEDIA FOR VARYING PERIODS AT −4° C. AS MEASURED BY THE LENGTH AND OVEN-DRY WEIGHT OF TOP GROWTH PRODUCED DURING THREE WEEKS OF RECOVERY IN THE GREENHOUSE. EXPERIMENT II. SERIES OF DECEMBER 14, 1935, TO FEBRUARY 5, 1936

DAYS STORED	ICE		CO_2 FLOWING		AIR FLOWING		N_2 FLOWING	
	WEIGHT	LENGTH	WEIGHT	LENGTH	WEIGHT	LENGTH	WEIGHT	LENGTH
	gm.	*cm.*	*gm.*	*cm.*	*gm.*	*cm.*	*gm.*	*cm.*
3	0.331	.....	0.440	.....	0.440	.....	.....	.....
9	0.580	.....	0.590	.....	.....	.....	.....	.....
17	0.400	.....	0.630	.....	.....	.....	0.625	.....
23	0.378	.....	0.472	.....	.....	.....	.....	.....
28	0.211	4.54	0.365	7.65	0.614	11.37	0.442	8.69
35	0.223	1.48	0.270	2.22	0.534	4.88	0.464	4.64
53	0.005	0.52	0.230	4.21	0.343	5.66	0.510	7.66

freezing at −4° C. under any of the conditions of surrounding media not in excess of ten days' duration. The growth for three weeks of the unfrozen check sample of plants before storage was much less than those stored in ice for 10 days and then allowed to recover for three weeks. More optimum light conditions in the greenhouse during the latter three weeks' recovery period may account, in part, for the increase. In any case, it indicates that the factors associated with the duration of the ice, rather than direct effects of the ice itself, are harmful.

In this experiment, as well as in Experiment I, the medium of ice was by far the most injurious. With 53 days of storage in ice nearly all the plants died. The injury curve for the flowing carbon dioxide follows that of ice but the injury was less intense. The plants held in flowing air and

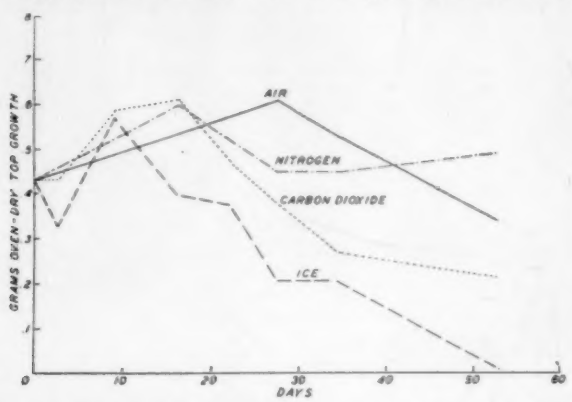

FIG. 3. Weight of oven-dry top growth of hardened Turkestan alfalfa produced in 3 weeks of greenhouse recovery after storage at −4° C. in ice and flowing gases. Experiment II. Series of December 14, 1935, to February 5, 1936.

flowing nitrogen were only slightly injured during this period but at the end of 53 days, the plants in air showed greater injury than those in nitrogen. In all probability this was due to more severe desiccation since the rate of the air flow was greater than that of nitrogen.

The trials of experiment I and II indicate that injury is intensified primarily by the *type* and *duration* of the surrounding media. This appears to be associated with accumulations and increasing concentrations of carbon dioxide and other products of aerobic and anaerobic respiration. The diffusion of carbon dioxide and other respiratory products away from living cells of plants tightly incased in ice, is definitely inhibited by the ice itself and it would appear that, in time, concentrations of considerable magnitude would result in serious injury. None of the surrounding media used in experiments I and II caused such intense and such rapid injury as did ice, and in no case would the diffusion of respiratory products be inhibited more completely. Further investigations of such relationships are incorporated in experiments III and IV.

Experiment III

Methods and procedure

To provide a series frozen at −4° C. for comparison with a series stored in water at 1° C. in experiment IV, a second lot of hardened Turkestan alfalfa plants was dug from the field on January 11, 1936. These were prepared as previously and subjected to −4° C. with various surrounding media similar to those in experiment II, namely, flowing air, flowing nitrogen, flowing carbon dioxide, stagnant nitrogen, and ice.

The recovery of these series as measured by the top growth produced

TABLE III

Recovery of hardened alfalfa plants after having been stored in various media for varying periods at −4° C. as measured by weight and length of the top growth produced during three weeks under greenhouse conditions. Experiment III. Series of January 11, 1936, to March 11, 1936

Days stored	Air flowing		CO_2 flowing		N_2 stagnant*		Ice	
	Weight	Length	Weight	Length	Weight	Length	Weight	Length
	gm.	*cm.*	*gm.*	*cm.*	*gm.*	*cm.*	*gm.*	*cm.*
3	0.678	13.61	0.569	10.76	.....	.....	0.437	9.3
10	0.542	11.85	0.423	8.76	.....	.....	0.287	4.28
23	0.488	8.26	0.455	8.70	.....	.....	0.170	2.36
31	0.635	12.08	0.210	4.91	0.325	7.44	0.002	0.9
38	0.510	6.23	0.370	5.37	.....	.....	0.040	0.55
45	0.705	10.34	0.200	7.26	0.260	4.38	0.0	0.0
53	0.540	10.39	0.215	5.41	0.110	2.25	0.0	0.0
60	0.413	4.33	0.010	0.92	0.100	2.20	0.0	0.0

* Test tubes were stoppered.

during three weeks of greenhouse recovery is presented in table III.[2] Figure 4 shows graphically the recovery as measured by oven-dry weight of top

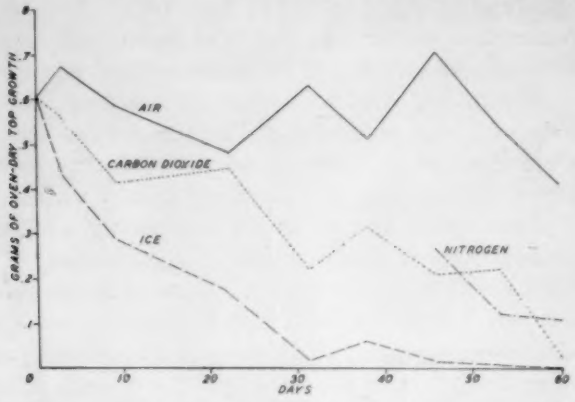

FIG. 4. Weight of oven-dry top growth of hardened Turkestan alfalfa produced in 3 weeks of greenhouse recovery after storage in ice, flowing air, flowing carbon dioxide, and stagnant nitrogen at −4° C. Experiment III. Series of January 11 to March 11, 1936.

growth produced during three weeks of favorable growing conditions in the greenhouse.

In this, as in previous trials, samples of alfalfa plants incased in ice show the greatest injury in the shortest time. Those stored in test tubes of flowing carbon dioxide are next in order but injury was very slight with storage in the medium of flowing air (fig. 4). With 60 days of storage all the plants in ice were dead and nearly all those in flowing carbon dioxide were dead. Those stored in stoppered tubes of nitrogen had slightly better survival but the plants were very much weakened after 60 days of storage.

The photograph (fig. 5) taken after three weeks of greenhouse recovery of the samples of plants removed after 31 days of storage shows a vigorous recovery of the plants frozen in flowing air and flowing nitrogen (both of which would reduce the accumulation of carbon dioxide and other respiratory products), but growth was retarded slightly (and later, severely) for those plants frozen in stoppered tubes of nitrogen where respiratory products could accumulate, and very much for those plants frozen in a stream of carbon dioxide. The plants frozen in ice were either dead or so seriously injured that very little or no top growth was produced.

In these trials and those of experiments I and II, it is probable that with the absence of oxygen in the surrounding gaseous media, the products

[2] Because of circumstances of an accidental character the data on flowing nitrogen is omitted except for results obtained after 31 days of storage which are shown photographically in figure 5.

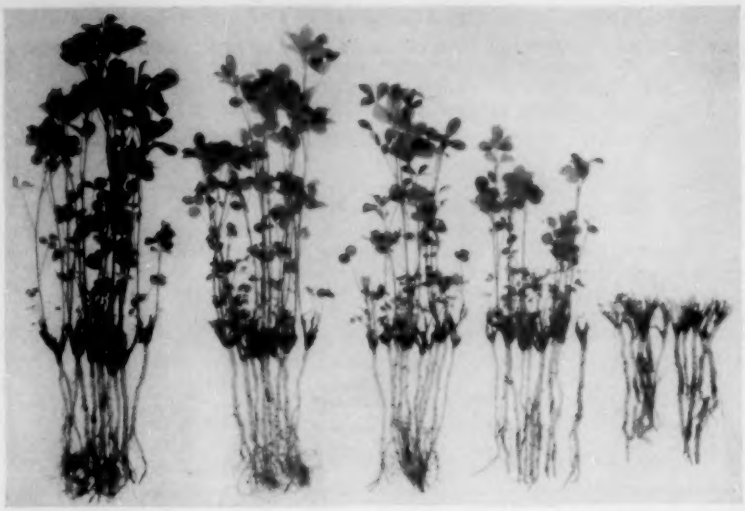

FIG. 5. Turkestan alfalfa plants after 3 weeks of recovery in the greenhouse. The plants (reading from left to right) had been stored at −4° C. for 31 days (January 11 to February 11, 1936) in flowing air, flowing nitrogen, stoppered test tubes of nitrogen, flowing carbon dioxide, and ice.

of anaerobic respiration become sufficiently concentrated to be toxic. Such harmful concentrations do not occur as rapidly in test tubes which provide for some diffusion as they do with ice which inhibits diffusion from the tissues almost completely.

Experiment IV

METHODS AND PROCEDURE

On January 11, 1936, hardened Turkestan alfalfa roots were obtained from the same field source as those in experiments II and III. They were trimmed as before and arranged in uniform ten-plant samples in Pyrex test tubes. Individual samples were surrounded at 1° C. with the following media: Tap water in stoppered test tubes, tap water through which carbon dioxide was bubbled, tap water through which air was bubbled, stagnant air, and stagnant nitrogen. After various periods of storage, ten-plant samples from each treatment were planted in quartz sand in the greenhouse for recovery.

RESULTS WITH STORAGE ABOVE FREEZING

The dry weight and length of tops produced during the recovery period following the treatments at 1° C. are presented in table IV and the data are comparable with those in table III. Unfrozen water produced the same detrimental effect as ice and the recovery of the plants stored in water closely

TABLE IV

RECOVERY OF HARDENED ALFALFA PLANTS AFTER STORAGE IN VARIOUS MEDIA FOR VARYING PERIODS AT 1° C. AS MEASURED BY THE WEIGHT AND LENGTH OF TOP GROWTH PRODUCED IN THREE WEEKS UNDER GREENHOUSE CONDITIONS. EXPERIMENT III. SERIES OF JANUARY 11, 1936, TO MARCH 11, 1936

Days stored	In water						No water	
	Air*		CO_2*		Stoppered		N_2 stagnant	
	Weight	Length	Weight	Length	Weight	Length	Weight	Length
	gm.	*cm.*	*gm.*	*cm.*	*gm.*	*cm.*	*gm.*	*cm.*
3	0.747	14.11	0.679	12.62	0.789	14.86	.....	.....
10	0.598	14.30	0.471	11.53	0.712	15.23	.....	.....
23	0.170	4.10	0.01	1.6	0.115	3.04	.....	.....
31	0.110	2.64	0.0	0.0	0.001	0.2	0.50	11.20
38	0.440	5.13	0.001	0.2	0.125	2.88	.....	.....
45	0.380	5.19	0.0	0.0	0.001	1.2	0.45	7.03
53	0.120	2.59	0.0	0.0	0.0	0.0	0.220	6.30
60	0.162	3.88	0.0	0.0	0.002	2.0	0.487	7.50

* Bubbled through test tubes of water.

resemble those of plants stored in ice but water saturated with carbon dioxide caused the greatest harm and all of the plants were severely injured in 23 days. Plants in stoppered tubes of water were nearly all dead in 31 days, while those in tubes of water with air bubbling through it were alive and vigorous at the end of 60 days. Plants stored in stagnant nitrogen but without water were healthy and vigorous at the end of 60 days.

In most cases, the injury occurred in the crowns. Often the crowns were dead, while the roots of such plants appeared uninjured. Especially was this true of the plants stored in water saturated with carbon dioxide. Field studies of GRABER (**2**) in 1922 showed that only the crowns and upper portions of the roots were injured and that the older thickened rhizomes of the crown were often dead, while the young rhizomes diverging from them were alive and apparently not seriously injured. Such plants, of course, eventually died, but the young rhizomes grew until warm weather and desiccation killed them. Combining these observations with those of experiment III, it would seem that ice-sheet injury under field conditions may be due, in part, to accumulations of carbon dioxide where the ice-sheet contacts the plants and that the older rhizomes are more susceptible to such injury. In these experiments, however, only young plants were used. The observations on such plants frozen at $-4°$ C. in ice for 35 days in experiment II and for 23 days in experiment III showed a noticeably higher degree of injury of crowns than of the roots, although in several instances the entire root was killed and decomposed to a pulpy consistency. This suggests that the crowns may be more sensitive to such injury or that the

carbon dioxide and other respiratory products may accumulate there in greater abundance.

The survival of hardened alfalfa plants with ice contacts appears to be related to the time of removal of the plants from their field environment. Plants removed on December 14, 1935, were injured by ice duration to a much less degree than plants given the same treatment after removal from the field on January 11, 1936. This may be of significance but an explanation will become available only with further trials.

Discussion

The authors have not found any literature dealing with the fundamental aspects of injury to alfalfa or other plants from external contacts with ice. While the exact mechanism of this type of cold injury is unknown the results obtained throw some light on some of the factors conditioning ice injury. These experiments were planned to eliminate direct injury from low temperatures so that the influences associated with the media immediately surrounding the plants could be ascertained. Aside from carbon dioxide, none of the surrounding media appeared to be directly injurious.

The interpretation of results obtained in the trials where gaseous and liquid media were confined in test tubes of limited capacity, would be incomplete without an appreciation of the fact that the daily production of carbon dioxide and other respiratory products by dormant plants would gradually increase the concentration of such products in the media and in the plant. The time factor of injury is probably associated with such increasing concentrations of carbon dioxide and other toxic materials of aerobic and anaerobic respiration. More than anything else this may explain the rapid rate of injury sustained by plants frozen in ice. Ice inhibited the removal, escape, and external diffusion of carbon dioxide and other products of aerobic and anaerobic respiration. True, ice excluded oxygen but the exclusion of this element does not seem to be an immediate cause of injury or death. Before the absence of oxygen became a lethal factor, it appears that concentrations of carbon dioxide and other respiratory products resulting from the incasement of the plant in ice, became the lethal factors. Plants stored in stoppered test tubes of nitrogen where oxygen was absent but where some diffusion of carbon dioxide could prevail, were injured less rapidly and much less intensely than plants frozen in ice.

That the removal of carbon dioxide and other respiratory products is associated with factors which greatly reduce the injuries of storage, is shown by the results of all the trials of this investigation. Plants stored in stoppered test tubes of water, were injured as intensely and as rapidly as those incased in ice but plants stored in water through which air was bubbled, were scarcely injured. Plants stored for 113 days at $-3.5°$ C. in stoppered

tubes of air showed decidedly greater injury than those stored in tubes of flowing air. Plants frozen in a constant stream of carbon dioxide showed a degree and rate of injury of a magnitude approaching that occasioned by ice-sheets.

The most rapid injury occurred when plants were stored in water saturated with carbon dioxide. When external accumulations of carbon dioxide were in contact with the plants in water solution, the injury was much greater than external accumulations in the form of gas. Carbon dioxide was the only form of external media used in these trials which appeared to be directly harmful. The rapid injury by carbon dioxide in water solution was observed by FREE (3) in 1917. He reported that buckwheat roots in culture solutions through which carbon dioxide was circulated "sickened and wilted within a few hours and died within a few days."

Summary

The injuries sustained by hardened alfalfa plants frozen and maintained in ice at harmless temperatures, appear to be due to internal accumulations of the toxic products of aerobic and anaerobic respiration. Ice, contacting the root and crown, inhibits the diffusion of carbon dioxide and concentrations of it and other respiratory products may increase rapidly, becoming highly injurious and ultimately lethal.

The degree and rate of injury of hardened alfalfa plants during various periods of storage was, in all cases, closely associated with conditions of the surrounding media which affected the removal of the respiratory product, carbon dioxide. Dormant alfalfa plants stored at non-injurious temperatures of −3.5° C., −4° C., and 1° C. for varying periods in test tubes of external media which removed carbon dioxide or, at least, prevented its accumulation, such as flowing air, and air bubbled through water, and flowing nitrogen, had a much higher survival and sustained much less injury and a much lower rate of injury than those confined for similar periods in ice and in test tubes of surrounding media, such as still water, still nitrogen, flowing carbon dioxide, and especially carbon dioxide bubbled through water.

The formation of ice about and in contact with the plant did not result in direct injury. Only after seven to ten days' duration of the ice were injurious effects manifested but after twenty days a high rate of mortality prevailed. Plants immersed in test tubes of water at 1° C. were injured at approximately the same rate and degree as those frozen in ice at −4° C. Where carbon dioxide was bubbled through water, however, such saturation reduced the survival much more rapidly and injuries were more intense than those sustained from ice contacts. Carbon dioxide was the only one, of the several gaseous media used, that appeared to be directly toxic to

plants and it was much more injurious when in solution than in the gaseous forms.

The exclusion of oxygen did not appear to be an initial cause of injury or death but may be associated with injuries resulting from the accumulations of the products of anaerobic respiration.

The writers wish to express their appreciation to Dr. B. M. DUGGAR and Dr. W. E. TOTTINGHAM for their helpful suggestions during the course of these experiments.

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LITERATURE CITED

1. BUGAEVSKII, M. F., and ZITNIKOVA, K. S. Choking winter wheat plants under an ice crust. (Trans. Title), Zbirn. Prac. (Rob.) Agrofiziol. **2**: 103–105. 1936.
2. GRABER, L. F. Twenty-first annual report of the Wisconsin Agricultural Experiment Station. p. 40. 1923.
3. FREE, E. E. The effect of aeration of the growth of buckwheat in water culture. Johns Hopkins Cir. 293. p. 198. 1917.

THE RELATIVE RATES OF DESTRUCTION OF PECTIN IN MACERATES OF VARIOUS CITRUS FRUITS[1]

M. A. Joslyn and A. Sedky

(with nine figures)

Introduction

The natural pectins are believed to consist of long, open, unbranched chains of galacturonic acid with an occasional arabinose and galactose molecule. The free carboxyl groups of the galacturonic acid are partially or wholly esterified with methyl alcohol, and an occasional hydroxyl group is esterified with acetic acid [Bonner (**4**), Branfoot (**5**), Freudenberg (**7**), Gaddum (**8**), Kertesz (**13**), Myers and Baker (**16**), Norman (**18, 19**), Ripa (**22**)]. The differences found among the natural pectins are believed to arise from differences in the extent of esterification, and in the extent of polymerization. The view that pectins originate phytochemically by the progressive oxidation of the sixth carbon atoms of the galactose residues occurring in galactans, followed by decarboxylation has been recently questioned by the finding of Hirst and Jones (**10**) that the araban found in close association with the pectic acid of the peanut is composed entirely of the arabofuranose residues instead of the expected arabopyranose residues.

On the assumption that the pectin molecule consists of galacturonic acid, galactose, arabinose, acetyl, and methoxyl units the pectic enzymes should number at least seven specific enzymes acting upon the possible linkages between these units. In addition we would expect the presence of enzymes of initial attack differentiated by their ability to attack pectins of different degrees of polymerization. Accepting "protopectinase" as such an enzyme, there are at present known but four specific enzymes (**13**) of which pectin methoxylase has been most widely studied.

The complexity of the pectins, the diversity of the enzymes acting upon them and the difficulty of obtaining either pure pectic enzymes or substrates of definite constitution and configuration have complicated this field. It has been only recently that systematic studies on fairly well defined substrates were begun by Ehrlich (**6**) and independently by Kertesz (**13**). The separation of the various components of the pectinase complex, their purification and investigation is yet to be undertaken. Rothschild (**23**) pointed out in this connection the possibility of separating "pectolase" from "pectase" by making use of the different rates at which these are destroyed in acid and alkaline solutions.

[1] Presented at the 23rd annual meeting of the Pacific Division, A.A.A.S., Stanford University, June 29, 1939.

Although some investigations were made by GADDUM (**6**) on the effect of maturity and fruit type on the chemical composition and physical properties of the pectins extracted from the albedo and pulp of citrus fruits, by HARVEY and RYGG (**9**) and by RYGG and HARVEY (**24**) on changes occurring in the pectic constituents of the peel of citrus fruit, no investigations on the nature and relative activity of citrus pectic enzymes have been reported. The citrus pectins are known to differ from each other and from those of other fruits in chemical composition and degree of polymerization. SVEDBERG and GRALEN (**27**) reported that the pectins from apples, pears and plums have approximately the same molecular weight while that from oranges was of definitely higher molecular weight (40,000–50,000). NORRIS (**20**) found that the pectic substances in oranges were less methoxylated than those found in lemons by NORMAN (**17**). Differences between commercial apple and citrus pectins were reported by STUEWER *et al.* (**26**). It has been shown, particularly by HIRST and co-workers at Birmingham (**2, 11, 12**), that the araban content of pectins may be reduced to negligible amounts by special methods of preparation so that arabinose need no longer be considered as an essential constituent of pectin. The precise nature of the galactan portion is not known (*cf.* NORRIS (**21**)) although strong evidence has been adduced to show that the fundamental feature of the chemical structure of both the pectic acid from strawberries and from citrus fruits consists of a chain of pyranose d-galacturonic acid residues linked together through positions 1 to 4, BEAVEN and JONES (**3**), SMITH (**25**). LEVENE *et al.* (**15**) believe the linkage is through the fifth carbon atom. This change in constitution of pectin reduces the number of enzymes in the pectinase complex. There is particular need at present for purification of the components of the pectinase complex as an aid to the study of the physiology and chemistry of pectins.

To obtain some information on the activity of the pectic enzymes present in citrus fruits, an investigation was made of the rate of decomposition of pectin in macerated tissues under various conditions. In preliminary investigations it was found that pectin destruction in oranges occurred at a very different rate from that in apple or cucumber tissues (fig. 1). In apple

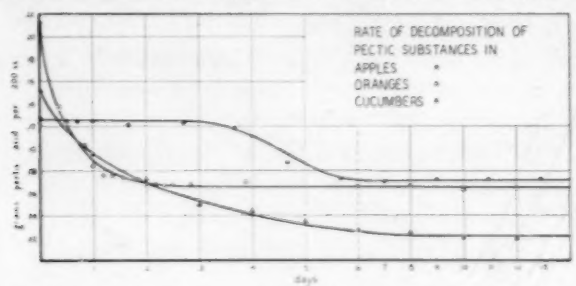

FIG. 1. Rate of decomposition of pectic substances in apples, oranges, and cucumbers.

the total pectin remained essentially constant for four days, then rapidly decreased to about two-thirds of its original value and remained at that level. In oranges the total pectin rapidly decreased to about one-third of its original value in 24 hours and then remained essentially constant, while the pectin content of cucumbers decreased rapidly and continually, reaching a very low level in about 6 days. The constant level in oranges and apples may be ascribed either to the presence of residual pectin unattacked by the enzymes present, or to the decay of the enzymes.

Materials and methods

The data shown in the graphs of figure 1 were obtained with Newton Pippin apples removed from cold storage in July 1937, fresh Valencia oranges and cucumbers. The macerates had the following initial pH values: 3.8, 4.5, and 5.8, respectively and a final pH of 3.5, 4.1, and 4.3, respectively.

The rates of destruction of pectin in orange, grapefruit, and lemon macerates were determined at their natural pH and in macerates adjusted to pH 2.5, 3.5, and 4.5, respectively and these data are presented here.

Preparation of samples

Valencia oranges, Marsh grapefruit, and Eureka lemons purchased early in the fall on the market, were peeled, the peel and pulp group ground separately, mixed together and 0.3 per cent. of sodium benzoate added as preservative. The time required for the preparation of the ground fruit was approximately two hours. The pH was then determined and, four hours after this determination, the entire amount of each fruit was divided into three lots; one of these was left at its original pH (3.5 for grapefruit and oranges and 2.5 for lemons) and the other two lots adjusted to give the range of 2.5, 3.5, and 4.5. Concentrated citric acid was added to obtain a lower pH and sodium hydroxide to obtain a higher pH. Immediately, and at definite intervals thereafter 50-gram samples were withdrawn after thorough mixing and boiled with 400 ml. of water for one hour to inactivate the enzymes and extract the pectin present, water being added periodically to maintain a constant volume. Extracts were then made up to 500 ml. and filtered. The appearance of the extracts shown in figure 2 illustrates the marked changes in pectin content (cloudy samples containing most).

The pectins present in 200-ml. aliquots of the filtrate were precipitated and determined as pectic acid (digalacturonic acid) by the Wichman-Chernoff procedure (**1**). On the assumption that the mineral content in all the samples withdrawn from a given lot of fruit is constant, the ignition of the pectic acid precipitate was omitted. In three of the series of tests the alcohol precipitate was determined, also, as a measure of gums, pectins, etc.

FIG. 2. Appearance of pectin extracts from crushed oranges, grapefruit, and lemons held at their natural pH value.

In the course of these experiments, the vacuum oven at 70° C. and the hot air oven at 100° C. were compared in the drying of pectic acid precipitate in some determinations, using extracts from oranges. The period of drying in the vacuum oven was approximately 24 hours, while that for the hot air oven was 8 hours. A comparison between the values obtained is given in table I.

It is evident that there is but a slight difference between the two methods of drying. The difference can be neglected, particularly in studies which

TABLE I

COMPARISON OF DRYING BY VACUUM OVEN AND BY HOT AIR OVEN

TIME OF STORAGE BEFORE DETERMINATION	VACUUM OVEN	HOT AIR OVEN
	PECTIC ACID PER 200 ML.	PECTIC ACID PER 200 ML.
hr.	*gm.*	*gm.*
0	0.214	0.213
4	0.185	0.183
8	0.157	0.156
12	0.127	0.127
14	0.119	0.118
22	0.102	0.102

are comparative in purpose rather than quantitative, therefore it is possible to use the hot air oven for drying pectic acid precipitates without seriously affecting the results.

CHANGES IN PH VALUE

Determinations of the pH values of the fruit extracts at the initial and final stages were made and the values obtained are given in table II.

TABLE II

CHANGES IN PH VALUES DURING THE EXPERIMENTS

PH	ORANGES			GRAPEFRUIT			LEMONS		
		NATURAL			NATURAL		NATURAL		
Initial ...	2.5	3.5	4.5	2.5	3.5	4.5	2.5	3.5	4.5
Final	2.7	3.8	4.1	2.8	3.7	4.2	2.8	3.9	4.1

It is evident that the pH increased slightly in all cases except for the sample brought initially to pH 4.5. In the latter the pH decreased to 4.1.

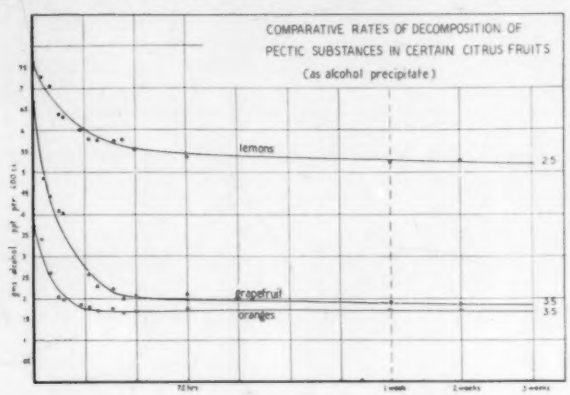

FIG. 3. Comparative rates of decomposition of pectic substances in certain citrus fruits. Expressed as alcohol precipitate.

The increase in pH is probably due to a change in the nature of the colloidal buffering substances present, while the decrease may be due largely to formation of free pectic acids by demethoxylation. The changes in pH, however, are not very large and may be neglected in view of the other changes involved. The results may be assumed to have been obtained at substantially constant pH value.

Comparison of citrus fruit extracts at their natural pH value

The rate of change in amount of alcohol precipitate obtained (by the A.O.A.C. procedure (**1**)), from samples of oranges, grapefruit, and lemons is shown in table III and in the corresponding graph (fig. 3). The data for

TABLE III

Rates of change in pectins of oranges, grapefruit and lemons determined as alcohol precipitate

Time of storage before determination	Grams alcohol precipitate per 200 ml. of filtrate		
	Oranges	Grapefruit	Lemons
hr.	*gm.*	*gm.*	*gm.*
0	0.375	0.691	0.775
4	0.343	0.488	0.728
8	0.263	0.442	0.705
12	0.205	0.433	0.640
14	0.199	0.408	0.633
22	0.185	0.403	0.598
26	0.179	0.262	0.581
30	0.175	0.229	0.577
38	0.176	0.226	0.572
42	0.169	0.200	0.576
48	0.170	0.209	0.552
72	0.173	0.214	0.539
168	0.174	0.190	0.527
336	0.166	0.186	0.532

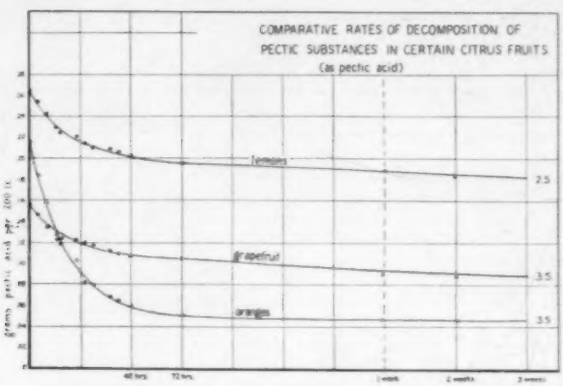

Fig. 4. Comparative rates of decomposition of pectic substances in certain citrus fruits (as pectic acid).

the pectin content determined as pectic acid are given in table IV and are represented by the graph shown in figure 4.

TABLE IV

RATES OF CHANGE IN PECTINS OF ORANGES, GRAPEFRUIT AND LEMONS DETERMINED AS PECTIC ACID

TIME OF STORAGE BEFORE DETERMINATION	GRAMS PECTIC ACID PER 200 ML. OF FILTRATE		
	ORANGES	GRAPEFRUIT	LEMONS
hr.	*gm.*	*gm.*	*gm.*
0	0.214	0.157	0.264
4	0.185	0.146	0.254
8	0.157	0.134	0.242
12	0.127	0.128	0.229
14	0.119	0.127	0.225
22	0.102	0.122	0.220
26	0.082	0.120	0.215
30	0.079	0.118	0.211
38	0.068	0.114	0.210
42	0.065	0.110	0.205
48	0.060	0.108	0.204
72	0.051	0.106	0.195
168	0.047	0.093	0.188
336	0.046	0.087	0.184

EFFECT OF pH ON THE RATE OF DECOMPOSITION OF CITRUS PECTINS

The changes in pectins observed in macerates adjusted to varying pH values are shown in table V and figures 5, 6, 7, 8, and 9.

TABLE V

CHANGES OF PECTIC SUBSTANCES IN FRUITS AT VARYING pH VALUES

TIME OF STORAGE BEFORE DETERMINATION	GRAMS PECTIC ACID PER 200 ML.								
	ORANGES			GRAPEFRUIT			LEMONS		
hr.	pH:—2.5	3.5	4.5	2.5	3.5	4.5	2.5	3.5	4.5
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
0	.....	0.214	.....	.....	0.157	.....	0.264	.....	.....
4	0.185	0.185	0.185	0.146	0.146	0.146	0.254	0.254	0.254
8	0.163	0.157	0.164	0.138	0.134	0.130	0.242	0.247	0.240
12	0.135	0.127	0.130	0.134	0.128	0.126	0.229	0.232	0.219
14	0.128	0.119	0.120	0.132	0.127	0.124	0.225	0.224	0.208
22	0.120	0.102	0.112	0.127	0.122	0.117	0.220	0.206	0.200
26	0.107	0.082	0.100	0.120	0.120	0.117	0.215	0.200	0.193
30	0.100	0.079	0.094	0.122	0.118	0.115	0.211	0.200	0.183
38	0.093	0.068	0.085	0.120	0.114	0.108	0.210	0.187	0.181
42	0.087	0.065	0.081	0.118	0.110	0.105	0.205	0.186	0.180
48	0.079	0.060	0.073	0.117	0.108	0.109	0.204	0.183	0.179
72	0.065	0.051	0.060	0.112	0.106	0.099	0.195	0.176	0.175
168	0.065	0.047	0.054	0.107	0.089	0.087	0.188	0.172	0.172
336	0.067	0.046	0.054	0.096	0.087	0.084	0.184	0.164	0.165
504	0.061	.....	0.053	0.092	.....	0.069	.....	0.161	0.158

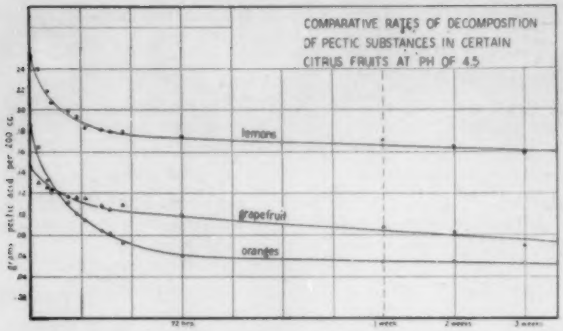

FIG. 5. Comparative rates of decomposition of pectic substances in certain citrus fruits at pH 4.5.

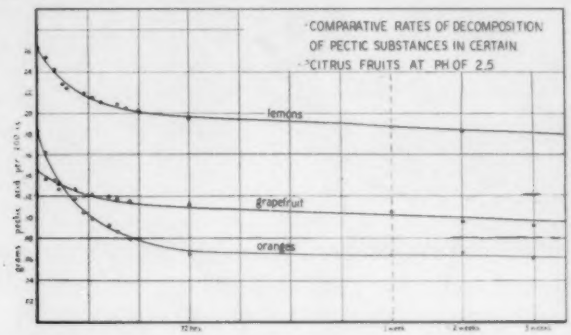

FIG. 6. Comparative rates of decomposition of pectic substances in certain citrus fruits at pH 2.5.

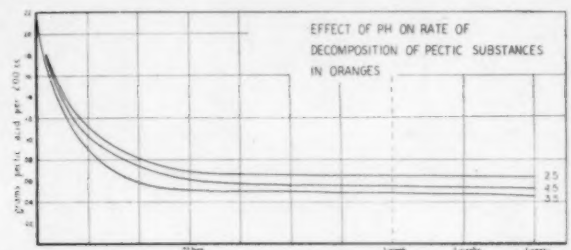

FIG. 7. Effect of pH on rate of decomposition of pectic substances in oranges.

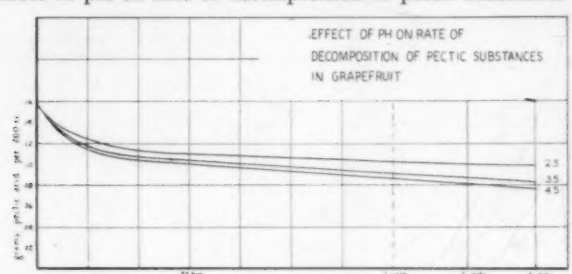

FIG. 8. Effect of pH on rate of decomposition of pectic substances in grapefruit.

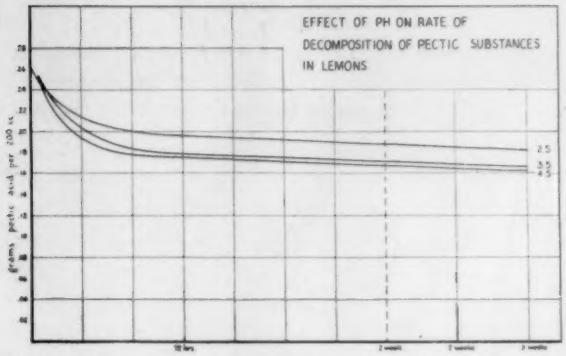

FIG. 9. Effect of pH on rate of decomposition of pectic substances in lemons.

Discussion

From the data shown in tables III and IV and the corresponding graphs (figs. 3 and 4) it is seen that the amount of soluble pectin in lemons is greater than that in oranges, and the amount in oranges exceeds that in grapefruit. A decrease in the amount of soluble pectin present in all three fruits occurs with increase in time of storage. It is of interest to note that this decrease is reflected in the extent of clarification of the filtrates from the different fruits as shown in figure 2.

On successive filtrates from each fruit at the natural pH value, there was marked clarification which was very rapid in oranges and more gradual in both grapefruit and lemons. In lemons the turbidity persisted throughout. The rate of decrease in the amount of unaltered pectic substance is shown by the graphs given in figures 3 and 4. It may be observed that a similar curve is obtained for each fruit; and that the rate of decrease in alcohol precipitate follows that of pectic acid. These graphs also show that for all fruits the initial rapid loss of soluble pectic substances occurred in approximately the same time.

In general, the decomposition of pectic substances measured as alcohol precipitate shows the same trend as that determined as pectic acid. If, however, we compare the ratio of alcohol precipitate to pectic acid precipitate in the three fruits after different time intervals, we observe a great variation as shown in table VI.

The ratio of alcohol precipitate to pectic acid remained approximately constant until after 22 hours, at which time most of the decomposable pectin is lost. In oranges the ratio then increases, indicating that although the gums and other non-pectinous water-soluble alcohol-precipitable materials were hydrolyzed at the same rate as pectin initially, they were decomposed at a slower rate thereafter. In lemons the ratio remained practically constant, thus indicating, either that the alcohol precipitable

TABLE VI

RATIO OF ALCOHOL PRECIPITATE TO PECTIC ACID IN SUCCESSIVE DETERMINATIONS

TIME OF STORAGE BEFORE DETERMINATION	RATIO OF ALCOHOL PRECIPITATE TO PECTIC ACID		
	ORANGES	GRAPEFRUIT	LEMONS
hr.			
0	1.8	4.4	2.9
4	1.85	3.3	2.8
22	1.8	3.2	2.7
48	2.8	1.9	2.7
72	3.4	2.0	2.7
168	3.7	2.6	2.8
336	3.6	2.7	2.8

material was all pectin or that the gums were decomposed at the same rate as pectin throughout. In the case of grapefruit the evidence indicates a more rapid decomposition of gums than of pectins in the initial stages followed by a less rapid decomposition.

It is seen that the total amount of soluble pectic substances decomposed varied greatly in the three citrus fruits. While in oranges almost all of this substance was decomposed, in grapefruit only about half of it had undergone such decomposition, and in lemons the amount decomposed was only one-third of the total. The reasons for this difference in behavior of the three fruits can only be surmised. Either there are definite differences in the nature and activity of the pectic enzymes present or there are differences in the pectins present. Although, as will be shown, the difference in pH had a measureable effect, it did not account for all the differences observed.

In figures 5 and 6 are shown the comparative rates of decomposition of pectic substances in the three fruits at pH values 2.5 and 4.5 respectively. In addition to the observation that in all cases the rate of decomposition was greater in oranges than in grapefruit or lemons, a comparison of the two graphs shows a greater decomposition of the pectic substances in all the fruits at the higher pH value.

With oranges, figure 7 indicates that the pectic substances were more rapidly decomposed at pH 3.5 than at either pH 2.5 or 4.5. The effect of the change of pH is more noticeable during the first three days; after this, the rates of decomposition approach constancy, a phenomenon represented by the parallelism of the curves.

With grapefruit (fig. 8), although the rate of decomposition was comparatively slow, it is seen that a pH of 4.5 was favorable for the decomposition. There is a marked difference in the rate of decomposition at pH 2.5 and 4.5, although the difference was smaller in the comparison between the rate of decomposition at pH 3.5 and 4.5.

The same results were obtained with lemons with the exception that the

rate of hydrolysis of pectic substance in lemons was faster than in grapefruit (fig. 9).

It is of interest to note that the effect on the decomposition of increasing the pH of each fruit from 2.5 to 4.5 is reflected in the degree of clarification in the extracts. On successive filtrates from each fruit at the upper pH range, the same progressive clarification was observed. This clarification is more apparent at the higher pH than at the normal or at the lower. In all cases, extracts obtained from fruit maintained at the lower pH value showed greater turbidity.

A study of pectin methoxylase activity in citrus fruits (to be presented elsewhere) indicated that this behaved similarly to that found in tomatoes by KERTESZ (**14**), *i.e.*, that its optimum pH was close to neutrality. In extracts from oranges or in pectin solutions to which these extracts were added, there occurred a very rapid decrease in viscosity but only a gradual increase in acidity owing to demethoxylation. This may indicate that a depolymerization of the pectin molecule precedes demethoxylation.[2]

Summary and conclusions

The rate of decomposition of natural pectins by the naturally occurring pectic enzymes was studied in various fruits. In apples the total pectin remained essentially constant for a period of about four days and then rapidly decreased to about two-thirds of its original value and remained at that level. In oranges the total pectin rapidly decreased to about one-third of its original value in the course of about 24 hours and then remained essentially constant. In cucumbers the decrease in pectin was fairly rapid and continuous.

A comparison of the rate of hydrolysis in oranges, grapefruit, and lemons indicated that:

1. The natural pectins hydrolyze more rapidly in orange than in grapefruit. In lemons the decomposition was slowest of the three.
2. In all cases, the rate of hydrolysis approached the maximum at the end of one day, decreased rapidly during the next two days and then gradually diminished.
3. The decomposition of pectin in the fruits was accompanied by the clarification of their extracts.

The effect of varying the pH of the extracts from oranges, grapefruit, and lemons was shown to be as follows:

1. The decomposition of pectic substance was found to be faster at pH

[2] Z. I. KERTESZ (Pectic enzymes IV. Structural considerations in connection with the enzymic hydrolysis of pectin. Jour. Am. Chem. Soc. 61: 2544–2548. 1939.) in a recently published paper reported also that "when pectin solutions are enzymatically decomposed the viscosity decreases more rapidly than corresponds to the increase in reducing power."

3.5 for oranges, and at 4.5 for both grapefruit and lemons, than at the other pH values tested.

2. The rate of decomposition at pH 2.5 for all fruits was much slower than at higher ranges.
3. The greatest effect of varying the pH value was observed in the early stages of the experiment.

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LITERATURE CITED

1. Assoc. Offic. Agr. Chem. Official and tentative methods of analysis. Fourth edition. P. 324–325. 1935.
2. BEAVEN, G. H., HIRST, E. L., and JONES, J. K. N. Pectic substances. Part IV. Citrus araban. Jour. Chem. Soc. **1939**: 1865–1868.
3. ————, and JONES, J. K. N. Molecular structure of pectic acid. Chemistry E Industry **58**: 363. 1939.
4. BONNER, JAMES. The chemistry and physiology of the pectins. Bot. Rev. **10**: 475–497. 1936.
5. BRANFOOT, M. H. A critical and historical study of the pectic substances of plants. Food Investigation, Special Report no. **33**: 1–154. 1929. His Majesty's Stationery Office, London.
6. EHRLICH, FELIX. Zur Kenntnis der Pektolase. Enzymologia **3**: 185–199. 1937.
7. FREUDENBERG, KARL. Polysaccharides and lignin. Ann. Rev. Biochem. **8**: 81–112. 1939.
8. GADDUM, L. W. The pectic constituents of citrus fruits. Florida Agr. Exp. Sta. Bull 268. 1934.
9. HARVEY, E. M., and RYGG, G. L. Field and storage studies on changes in the composition of the rind of Marsh grapefruit in California. Jour. Agr. Res. **52**: 747–787. 1936.
10. HIRST, E. L., and JONES, J. K. N. Pectic substances. Part I. The araban and pectic acid of the peanut. Jour. Chem. Soc. **1938**: 496–505. 1938.
11. ————, and ————. Pectic substances. Part II. Isolation of an araban from the carbohydrate constituents of the peanut. Jour. Chem. Sec. **1939**: 452–453.
12. ————, and ————. Pectic substances. Part III. Composition of apple pectin and the molecular structure of the araban component of apple pectin. Jour. Chem. Soc. **1939**: 454–460.
13. KERTESZ, Z. I. Pectic enzymes. Ergeb. Enzymforsch. **5**: 233–250. 1936.

14. ————————. Pectic enzymes. II. Pectic enzymes of tomatoes. Food Res. **3**: 481–487. 1937.
15. Levene, P. A., Meyer, G. A., and Kuna, Martin. Preliminary note on the mode of union of the galacturonic acid residues in pectic acid. Science n.s. **89**: 370. 1939.
16. Myers, Philip B., and Baker, George L. Fruit jellies. VIII. The rôle of pectin. 4. The physico-chemical properties of pectin. Delaware Agr. Exp. Sta. Bull. 187. 1934.
17. Norman, A. G. Studies in pectin. III. The degree of esterification of pectin in the juice of the lemon. Biochem. Jour. **22**: 749–752. 1928.
18. ————————. Biochemistry of pectin. Sci. Prog. **24**: 263–279. 1929.
19. ————————. The biochemistry of cellulose, the polyuronides, lignin, etc. Oxford. 1937.
20. Norris, F. W. Pectic substances of plants. IV. The pectic substances in the juice of oranges. Biochem. Jour. **20**: 993–997. 1926.
21. ————————. Review on pectin in Ann. reports on the progress of chem. **35**: 362–365. The Chemical Society, London. 1939.
22. Ripa, R. Die Pektinstoffe. Braunschweig. 1937.
23. Rothschild, Hans. Studies on pectins. IV. Isolation of pectic enzymes. Enzymologia **5**: 359–364. 1939.
24. Rygg, G. L., and Harvey, E. M. Behavior of pectic substances and naringin in grapefruit in the field and in storage. Plant Physiol. **13**: 571–586. 1938.
25. Smith, F. Molecular structure of pectic acid. Chemistry E Industry, **58**: 363–364. 1939.
26. Stuewer, R., Beach, N. M., and Olsen, A. G. Pectin studies. II. Sugar-acid-pectin relationships and their bearings upon routine evaluation of apple pectin. Ind. Eng. Chem., Anal. Ed. **6**: 143–146. 1934.
27. Svedberg, The, and Gralen, Nils. Carbohydrates of well defined molecular weight in plant juices. Nature **142**: 261–262. 1938.

MOVEMENT OF ORGANIC SOLUTES IN THE SAUSAGE TREE, *KIGELIA AFRICANA*[1]

HARRY F. CLEMENTS

(WITH ONE FIGURE)

Introduction

In recent years, considerable emphasis has been placed on the concept that organic materials, assumed to be sugar, move *en masse* with the water in which they are dissolved through phloem tissues to points of storage or utilization. The mass flow hypothesis, as this concept has been labelled, was suggested independently by MÜNCH (**9**) and CRAFTS (**3**) in recent times, although HARTIG (**7**) in 1858 and SACHS (**10**) in 1865, proposed ideas somewhat similar. No attempt has been made, however, to subject this concept to an objective examination. It is the purpose of this paper to study the growth and development of the fruits of the sausage tree in light of this concept and to determine whether or not the hypothesis can explain the movement as observed in this plant.

The mass flow concept

Before presenting the data collected, it is perhaps necessary to re-state the mass flow concept. MÜNCH (**9**) proposed that the sugar solution moves through the sieve tubes of the phloem from the point of source (storage organ or photosynthetic tissues) to the point of utilization. CRAFTS (**3**) broadens the concept of the tissues involved to the whole phloem including sieve tubes, companion cells and parenchyma, and further proposes that the sugar solution in its movement traverses not only the lumina of the cells but the cell walls as well. No data are presented demonstrating either of these postulates except observations associated with the exudation of liquid material obtained from the phloem area when this tissue is cut. It has been assumed that this exudation represents movement in the *intact* plant. COOIL (**2**) has shown, however, that the only significance which can be attached to this exudation phenomenon is that it represents the readjustment of tissue tensions following the disruption caused by cutting the phloem as well as the associated parenchyma and xylem tissues. The fact that most species do not show exudation at all has been claimed by CRAFTS (**4**) to be due to the inadequate technique of the investigator. He shows that if any species is cut and the wounded portion dipped into water, streaming can be observed from the exposed surface and he assumes that this is a demonstration of streaming in the uninjured plant. Such streaming, however, may

[1] Published by permission of the Director of the Hawaii Agricultural Experiment Station as technical paper no. 74.

mean one of two other things: (1) that two materials of different specific gravities and viscosities have been mixed—the heavier one sinks and because of its greater viscosity gives the impression of streaming; (2) that exudation from the cut sieve tubes is induced as a result of dipping the exposed tissue in water, thereby causing a localized osmotic movement resulting in the displacement of sieve tube cytoplasm by the absorbed water entering by way of the parenchymatous cells associated with the sieve tubes. At any rate, the sugar solution is presumed to move through phloem tissue very much as the transpiration stream moves through the xylem.

The second point on which no pertinent data have been presented involves the concentration of the solution which moves. Although he presents no data obtained by chemical analysis showing the amount of sugar actually present in the exudate, CRAFTS assumes that at least two-thirds of the dry material collected in the exudate is sugar, and therefore that the phloem sap is at least a 10 per cent. sugar solution. SACHS (**10**), however, in 1865 showed that the dry matter in the sieve tube of cucurbit was largely nitrogenous in nature and that only a very small amount of sugar appears in the tube. In fact, this led him to propose that sugars did not move in the sieve tubes at all and that only nitrogenous compounds move there, while sugars moved in the associated parenchyma tissues. COOIL (**2**) recently completely substantiated these compositional observations. About one-third of the dry material of the exudate is simple protein, and sugar constitutes about 0.5 per cent. of the fresh weight.

The third point involves the time in the ontogeny of the phloem element at which this movement takes place or at least when it is most active. CRAFTS has shown by means of plasmolysis and dye absorption that as the sieve tube ages, it becomes more and more permeable. Whether any significance can be attached to these observations is beside the point. The significant point is that this is taken to mean that the sieve tube is essentially passive in the movement of material. Therefore, it seems clear that the old sieve tubes should be more effective in transport than the young tubes which are thoroughly alive, and therefore far from passive.

The fourth point of the hypothesis is that once the sugar solution reaches the point of storage or utilization, the water passes into the xylem system and thus circulates back through the plant. No data are presented to support the assumption that this normally occurs in plants.

Although there still remains the matter involving the movement of water in large quantities from xylem and mesophyll into the phloem, this particular point, although again shown clearly in diagrams, has not been shown to occur in plant tissues in volume sufficient to account for movement throughout the phloem. This point, however, is not cogent to this paper, and hence will not be pressed here.

Thus, briefly, the mass flow hypothesis states that a concentrated sugar solution (at least 10 per cent.) moves *en masse* through passive phloem tissues and the water, after dropping its sugar, returns by way of the xylem tissues.

Experiment

The fruits of the sausage tree (*Kigelia africana*, Bentham) are good subjects for this type of research. On each inflorescence, about eight fruits form, but abscission claims half of these. Four fruits may remain on a single floral stem and develop to maturity. There may be more than a hundred such clusters on a tree. At maturity, the floral stem may be 150 cm. long, hanging from the tree (fig. 1) supporting four large fruits, each one

FIG. 1. The fruits of *Kigelia africana* showing one cluster of four fruits.

of which may weigh five pounds or more. To be sure, many of the clusters have fewer than four fruits, especially since the tree is very rare in the northern hemisphere and souvenir hunters are numerous.

On June 9, 1939, the flowers were hand pollinated and on June 18, 1939, several clusters were selected for observations. The floral stem of one of these clusters was girdled. All the fruits of this cluster stopped growth and soon dropped. The usual interpretation would be that by breaking the continuity of the phloem food was stopped and hence the fruits dropped.

Another cluster of fruits was enclosed in a bag made of black paper. The object here was to prevent photosynthesis from taking place since the fruits are normally green when young. That the treatment was effective was indicated by the fact that the enclosed fruits were quite without green color. Thus, all of the food which this fruit consumed in growth and storage had to move into it via the floral stem.

Three other clusters were tagged but were without treatment. Two of these were interfered with by tourists and were abandoned.

Beginning June 18, 1939, and continuing at intervals until September 10, each fruit of each cluster was measured by determining its length and circumference. Since the fruits are solid and essentially round in cross-section, it is easy to get a fairly accurate measure of the volume. At the time each group of measurements was made, a fruit corresponding in size to those being measured was selected from the tree, measured, weighed, dried and again weighed. In this way, it was possible to determine the weight of the experimental cluster after each interval and also to calculate therefrom the increase in dry matter for each period.

Phloem samples from the floral stem were gathered at three intervals. The phloem area is very clearly marked and it was easy to remove the xylem tissues as well as the tissues external to phloem. Microscopic examination revealed that the tissue remaining was phloem only. These samples were dried and preserved for sugar analysis.

Results

Only the results obtained from the bagged cluster of fruits will be presented here. Actually, bagging made no difference in the development of the fruit. The unbagged cluster showed no significant difference over the bagged fruit. The record of the volume growth of the four fruits which were bagged are reported in table I.

Each time measurements were made, fruits comparable to those being measured were removed, and their volume, green wight, and dry weight obtained. From these data, the green weights and dry weights of the bagged fruits were calculated. These data are shown in table II.

Although no one has ever demonstrated the form in which organic carbon moves, it has generally been assumed to be sugar. Very probably some of the carbon enters a fruit as amino acids, but the total nitrogen content of these fruits is 1.41 per cent. of the dry weight. If the carbon asso-

TABLE I

VOLUME RECORD FOR ONE CLUSTER OF FOUR FRUITS

DATE OF MEASUREMENT	VOLUME TOTAL	INCREASE OVER PREVIOUS MEASUREMENT	INCREASE IN VOLUME PER DAY
	ml.	*ml.*	*ml.*
June 18, 1939	619	619	68*
June 25	2128	1509	215
July 2	4573	2444	349
July 9	6346	1772	253
July 15	7634	1287	214
July 21	8450	816	136
July 29	9355	905	113
August 9	9716	361	32
August 20	9871	154	14
August 29	9948	77	8
September 10	9979	30	2

* Calculated from the date of pollination, June 9, 1939.

ciated with this nitrogen moved as amino acids rather than sugar, the total amount of carbon thus moved would still be considerably less than 5.0 per cent. of the total carbon required for the fruit. Even though the possibility of translocatory compounds other than sugar exists, in this work the assumption that sugars are the important carbon carriers into these fruits will be maintained.

In order, therefore, to obtain a sugar value for the dry material which

TABLE II

GREEN AND DRY WEIGHTS FOR ONE CLUSTER OF FOUR FRUITS

DATE	GREEN WEIGHT PER ML.	TOTAL GREEN WEIGHT OF FRUITS	DRY MATTER	TOTAL DRY WEIGHT	INCREASE IN DRY WEIGHT	DAILY INCREASE IN DRY WEIGHT
	gm.	*gm.*	%	*gm.*	*gm.*	*gm.*
June 18, 1939	1.117	692	11.9	82.3	82.3	9.1
June 25	1.037	2207	11.9	262.8	180.5	25.8
July 2	0.933	4267	11.5	490.7	227.9	32.6
July 9	0.957	6073	11.3	686.3	195.6	27.9
July 15	0.957	7305	11.3	825.6	139.3	23.2
July 21	0.957	8086	11.3	913.8	88.2	14.7
July 29	0.913	8541	12.1	1033.5	119.7	14.9
August 9	0.890	8648	14.1	1219.3	185.8	16.9
August 20	0.883	8716	15.4	1342.3	123.0	11.2
August 29	0.887	8824	17.4	1535.5	193.2	21.5
September 10	0.879	8771	18.7*	1640.3	104.8	8.7

* On November 15, 1939, a fruit was taken from the tree. The dry weight had increased only 0.5 per cent. indicating that by September 10, growth was very nearly completed.

moved into the fruit, a total carbon analysis of the fruit was made.[2] This was found to represent 43.46 per cent. of the dry material. In table III, the total amount of carbon moving into the fruit each day is reported, and, granting that carbon moves into a fruit in the form of sugar, it is simple to convert the daily additions of carbon to the fruit into a hexose sugar equivalent. These values are also shown in table III.

TABLE III

DAILY GAIN IN DRY WEIGHT EXPRESSED AS CARBON AND AS HEXOSE SUGAR

DATE	DAILY GAIN IN DRY WEIGHT EXPRESSED AS	
	CARBON	HEXOSE SUGAR EQUIVALENT
	gm.	*gm.*
June 18, 1939	3.95	9.87
June 25	11.21	28.03
July 2	14.17	35.42
July 9	12.12	30.30
July 15	10.08	25.20
July 21	6.39	15.98
July 29	6.48	16.20
August 9	7.34	18.35
August 20	4.87	12.17
August 29	9.34	23.35
September 10	3.80	9.50

Thus, the figures given in the "hexose sugar" column represent the amount of sugar which each day moves into the fruit by way of the long floral stem. To this should be added the amount of sugar which is respired each day. Respiration measurements were not made during these studies, but a value could be arrived at from some similar types of fruits. On the basis of such calculations between two and three grams of sugar are respired each day. But since such values represent about 10 per cent. of the sugar moving into these fruits, the sugars used in the respiratory activity are ignored in this study. It is reasonable to assume, however, that respiration per unit volume is much higher in the young fruits than in the old fruits.

In summarizing this phase of the study, it is clear that by far the greatest increase in fruit volume is accomplished during the first five weeks, for it is during this period that the largest daily increments are observed. During this same period the daily increments of dry material are higher than those observed after the floral stem is more mature. During this early period, the tissues of the floral stem are young; in fact, the long stem (145 cm.), which for the most part is really a single internode, is still elongating. One would expect the metabolic rate of this tissue to be considerably higher than that

[2] The author is indebted to Mrs. RUTH YOSHIDA of the Soils Division for this determination.

during the later two-month period. Hence, it appears that the greatest deposition of carbohydrate material in the fruit occurs during the time when the phloem elements of the floral stem are young. This relationship is probably less coincidental than it is causal.

The next phase of this study involves a determination of the strength of the sugar solution which obtains in the phloem of the floral stem at the time of fruit development and, since the total amount of sugar necessary to provide the dry material actually observed moving into the fruits each day is known, it becomes a simple matter to determine the total volume of solution which would be required to move the sugar into the fruit were the mass flow hypothesis the correct interpretation of the mechanism involved.

On July 9 and again on July 15, during the peak of the fruit growth, and finally on September 10, phloem samples were taken from the floral stem by removing xylem tissues as well as the outer paranchymatous tissues. This separation was made easy by a ring of fiber groups just outside the phloem region. Microscopic examination showed the sample to be made up of phloem only. Stained preparations showed a large number of sieve tubes and companion cells, some sclerenchyma and a large amount of parenchyma. Microchemical tests for reducing sugars as well as sucrose revealed moderate concentrations in the inner phloem and somewhat lighter concentrations in the older phloem.

The tissue was dried, and analyzed for reducing sugars and total sugars. The results of this study are reported in table IV.

In the last column of table IV, the amount of sugar in 100 gm. of fresh material is calculated in terms of percentage solutions. Since it is assumed

TABLE IV

SUGAR CONTENT OF THE PHLOEM TISSUE*

DATE OF COLLECTION	DRY WEIGHT	REDUCING SUGARS		
		REDUCING SUGARS AS PERCENTAGE DRY WT.	AMOUNT OF SUGAR PER 100 GM. FRESH WT.	PERCENTAGE SOLUTION
	%	%	*gm.*	%
July 9, 1939	11.3	5.28	0.597	0.672
July 15	11.6	4.35	0.505	0.571
September 10	24.4	1.73	0.422	0.558
		TOTAL SUGARS		
July 9, 1939	11.3	14.00	1.582	1.78
July 15	11.6	13.11	1.521	1.71
September 10	24.4	11.38	2.777	3.67

* The author is indebted to Mr. BRUCE COOIL for these determinations.

by CRAFTS that the whole phloem area is involved in transport then presumably all the water in the phloem is mobile. Hence, the sugar present must be expressed on a solution basis. Reducing sugars which probably are the most important in transport are present in low concentrations. The reducing sugar values obtained here average 0.62 per cent. and correspond closely to the values obtained by COOIL (**2**) for the exudate of cucurbits but are vastly different from the 10 per cent. assumed by CRAFTS.

Now, it is hardly likely that the parenchyma of the phloem is even fractionally as active in translocation as the sieve tube, if at all. According to COOIL, the petiole tissues of a carbohydrate-high plant are found to be very high in sugar while in a nitrogen-high plant, the tissue sugars are low. Yet, the amount of sugar in the exudate of the phloem is about the same. This probably means that the sugar in the parenchyma is storage material. Sucrose is probably more of a storage sugar than a translocatory sugar. Despite these considerations, however, to give the benefit of all doubts to a favorable interpretation of the mass flow hypothesis, the amount of water required to move the sugar into the fruit during each day is calculated on the basis of the reducing sugar as well as the total sugar concentrations. The sugar values for the collections July 9 and 15 serve as the basis for these calculations, since these represent the period of maximum deposition in the fruit. Unfortunately, a collection of phloem tissue was not made on July 2.

TABLE V

AMOUNT OF SOLUTION NECESSARY TO TRANSPORT THE REQUISITE AMOUNT OF SUGAR INTO THE FRUITS PER DAY

DATE	GRAMS OF SOLUTION	
	0.62 PER CENT. SOLUTION	1.75 PER CENT. SOLUTION
	gm.	*gm.*
June 18, 1939	1592	564
June 25	4521	1602
July 2	5713	2024
July 9	4887	1731
July 15	4064	1440
July 21	2577	913
July 29	2613	926
August 9	2960	1049
August 20	1963	695
August 29	3766	1334
September 10	1532	543

It is, therefore, apparent (table V) that, during the time of maximum fruit development, enormous quantities of solution (5.7 liters of a 0.62 per cent. solution or 2.0 liters of a 1.75 per cent. solution) would have to be forced through the phloem each day. The cross-sectional area of the phloem

tissue was only 0.53 cm.², and the floral stem was 145 cm. long, not including the 15 cm. length of stem which connected the main floral branch to each individual fruit. In table VI, the amount of water required to move the necessary amount of sugar into the fruits is put on the basis of 1 cm.² of phloem cross-sectional area.

TABLE VI

RATE OF SOLUTION MOVEMENT REQUIRED PER SQ. CM. OF PHLOEM AREA

DATE	RATE OF SOLUTION MOVEMENT			
	AS 0.62 PER CENT. SOLUTION		AS 1.75 PER CENT. SOLUTION	
	PER DAY	PER HOUR	PER DAY	PER HOUR
	gm.	*gm.*	*gm.*	*gm.*
June 18, 1939	3009	125	1066	44
June 25	8545	356	3028	126
July 2	10797	449	3825	159
July 9	9236	384	3272	136
July 15	7681	320	2722	113
July 21	4871	203	1726	71
July 29	4939	205	1750	72
August 9	5594	233	1983	82
August 20	3710	154	1314	54
August 29	7117	296	2521	105
September 10	2895	120	1026	42

Thus, depending upon the strength of the sugar solution, the rate of solution movement at the peak of fruit development would have to be 159.4 or 449.1 gm. of solution per cm.² of phloem *per hour*. Now DIXON (5) in his studies of water transport in xylem tissues finds that a movement of 1.93 grams of H_2O per cm.² of xylem per hour was sufficient to account for the speeds of water transport in *Taxus*. FARMER (6) reports that the xylem of certain angiospermous trees delivers 20 ml. of water per hour per unit of cross-sectional area. It is hardly reasonable to believe that water movement in the phloem is many times faster than in xylem systems.

One further point remains to be shown. In table V is shown the daily amount of water necessary to move the requisite amounts of sugar into the fruit. It is necessary to follow this water and determine its ultimate disposition. Each day, according to the mass flow hypothesis, a certain volume of water moves through the floral stem into the fruits. Here the sugar is removed from the solution, and the water is then eliminated from the fruit to make room for more. According to the hypothesis, this water returns by way of xylem tissue. Some of the water, however, would remain to make up the daily volume increase and of course some is lost in transpiration. The amount lost in transpiration is small, since the fruits hang in the shade of the tree. According to measurements, transpiration accounts for less than 20

gm. per day per fruit and, in view of the large amount of water involved, can be ignored. The increases in volume are taken into account in table VII.

TABLE VII

AMOUNTS OF WATER RETURNING FROM FRUIT

DATE	AMOUNTS OF H_2O MOVED INTO FRUIT EACH DAY,—CALCULATED AS		VOLUME INCREASE EACH DAY	AMOUNT OF H_2O WHICH MUST RETURN VIA XYLEM	
	A 0.62 SOL.	A 1.75 SOL.			
	gm.	*gm.*	*ml.*	*Column 1 minus 3*	*Column 2 minus 3*
June 18, 1939	1592	564	69	1523	495
June 25	4521	1602	216	4305	1386
July 2	5713	2024	349	5364	1675
July 9	4887	1731	253	4634	1478
July 15	4064	1440	215	3849	1225
July 21	2577	913	136	2441	777
July 29	2613	926	113	2500	813
August 9	2960	1049	33	2927	1016
August 20	1963	695	14	1949	681
August 29	3766	1334	9	3757	1325
September 10	1532	543	3	1529	540

Thus, if the solution moves into the fruit as a 0.62 per cent. solution, 5.4 liters of water would have to return via xylem tissues each day (week ending July 2). If the solution were a 1.75 per cent. solution then 1.7 liters would have to return. Even the smaller figure involves an hourly average of 69.8 ml. The larger value would involve a backward movement of 223.5 ml. per hour. To test this point, a cluster of fruits was selected on July 9. A longitudinal cut was made through the bark of the floral stem, the xylem was carefully loosened, then cut, and the fruit end of the cut xylem was led out through the incision.

Thus, with the phloem intact, the water returning through the xylem should be intercepted at this point, and with such amounts as 69.8 ml. or 223.5 ml. per hour, the water should literally spout out especially since the radius of the xylem is about 1 mm. But after waiting three hours during late morning, with no sign of water coming out of the cut xylem, this project was discontinued.

At this point it is well to compare the findings of this paper with the assumptions underlying the mass flow hypothesis. The assumption that sugars move in a solution involves the movement of such tremendous quantities of water through poorly adapted tissues that it is inconceivable that any protoplasm no matter how permeable or passive could remain in the sieve tubes and other cells of the phloem. The necessary rates of movement are many times greater than have been observed in xylem tissues.

The assumption that the protoplasm of the conducting tissues is passive is not based on fact. In these studies there is a much greater movement of sugar during the early stages of floral stem development than there is later on. At this stage the tissues are young and certainly more active metabolically than later on. If the movement of sugar were correlated with passivity of protoplasm, it should occur late, and yet such is not the case.

The assumption that sugar in the phloem tissue exists as a 10 per cent. solution is a gross exaggeration; for the exudate of cucurbits as well as the phloem tissue of the sausage tree, the value is less than one per cent.

The assumption that the water which carries the sugar into the fruit returns by way of the xylem could not be verified in these studies.

Thus, it would appear that the mass flow hypothesis is hopelessly inadequate in accounting for the growth and development of the fruits of *Kigelia africana*.

On the other hand, it is much simpler to conceive of the sugar moving in solution but independently of the more or less static solvent. That this movement is associated with the activity of protoplasm is indicated by the fact that the greater part of the growth and development of the fruit takes place while the sieve tubes are young and at a higher metabolic level than later on. The amounts of sugar moving are so great that it seems that the living protoplasm expends energy in aiding the translocation of sugars (**1, 8**).

Summary

1. The growth of the fruits of *Kigelia africana* was followed from the day of pollination until maturity to determine the required rate of sugar movement into them.

2. An attempt was then made to explain this movement on the assumptions of the mass flow hypothesis.

3. The hypothesis was found to be inadequate in the following particulars:

a. Although a passive sieve tube cytoplasm is necessary to the hypothesis, the greatest movement of materials into the fruit was observed during the first five weeks of development.

b. Although the strength of the moving sugar solution has been assumed to be at least a 10 per cent. solution, in these studies the phloem tissue has a reducing sugar level of 0.62 per cent. and a total sugar level of 1.75 per cent.

c. No evidence could be obtained relating to the return of water from the fruit through xylem tissue even though enough time was allowed for the theoretical return of as much as 209 to 670 ml.

d. On the basis of the sugar content of the phloem tissue, to account for the observed movement of sugar into the fruit, such enormous quan-

tities of solution (10,797 ml. of a 0.62 per cent. or 3825 ml. of a 1.75 per cent. per day per cm.^{2} of phloem cross-sectional area) would have to move that it is inconceivable that any cytoplasmic organization would remain within the phloem tissue. In fact, the necessary speed of solution delivery into the fruit would be many times the capacity of xylem tissues.

4. The observed movement of sugar into the fruit is so great that it seems necessary to describe it as a function of the living protoplasm of the sieve tube which through its respiratory activity does work in the movement.

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LITERATURE CITED

1. CLEMENTS, HARRY F. Translocation of solutes in plants. Northwest Science **8**: 9–21. 1934.
2. COOIL, B. The significance of phloem exudate of *Cucurbita pepo* with reference to translocation of organic materials. (In press.)
3. CRAFTS, A. S. Movement of organic materials in plants. Plant Physiol. **6**: 1–42. 1931.
4. ———. The relation between structure and function of the phloem. Amer. Jour. Bot. **26**: 172–177. 1939.
5. DIXON, H. H. Transpiration and the ascent of sap. Macmillan. 1914.
6. FARMER, J. B. On the quantitative differences in the water conductivity of the wood in trees and shrubs. Proc. Roy. Soc. London **B90**: 218–250. 1918.
7. HARTIG, TH. Ueber den Herbstsaft der Holzpflanzen. Bot. Zeit. **16**: 369–370. 1858.
8. MASON, T. G., and MASKELL, E. J. Oxygen supply and the activation of diffusion. Ann. Bot. **50**: 455–499. 1936.
9. MÜNCH, E. Versuche über den Saftkreislauf. Ber. d. bot. Ges. **45**: 340–356. 1927.
10. SACHS, JULIUS. Lectures on the physiology of plants (Trans. by H. M. WARD). pp. 353–365. 1887.

DETERMINATION OF AMMONIA, GLUTAMINE, AND ASPARAGINE AMIDE NITROGEN IN PLANT JUICE[1]

FRANK S. SCHLENKER

(WITH ONE FIGURE)

The most generally used method for the determination of preformed ammonia in plant material is the aspiration of a sample with $Ca(OH)_2$, MgO, or 52 per cent. K_2CO_3. This technique was shown to include other than preformed ammonia nitrogen (**4**) for, with prolonged aeration, there was a continual evolution of ammonia, owing to the presence of unstable nitrogen compounds. PUCHER (**3**) reported that this was caused by the presence of amides.

This error was obviated in plant juice by the removal of ammonia by permutit, washing free from excess juice, treating with NaOH, and aspirating in the Van Slyke-Cullen ammonia apparatus.

CHIBNALL and WESTALL (**1**) and VICKERY, *et al.* (**5**), have shown that it is possible to determine glutamine and asparagine amide nitrogen in a mixture of the two amides by choosing the proper conditions for hydrolysis. In an attempt to collect a number of methods into a system for the analysis of plant juice it was thought worth-while to study the adsorption of amides by permutit, and to reinvestigate the use of permutit for the determination of ammonia.

Determination of ammonia

The action of 52 per cent. K_2CO_3 on plant juice under continued aeration causes a breakdown of the amides present. A typical curve for this, using beet juice, is shown in figure 1 (curve D), the break coming at the end of two hours.

An attempt was made to simulate this curve with a synthetic solution of the following composition: 0.0812 mg. NH_3–N, 1.5 mg. asparagine amide–N, 0.959 mg. glutamine amide–N and 1 ml. buffer[2] per 5-ml. sample. In figure 1, each curve, excepting curve D, represents the effect of aerating this mixture or variations of it, lacking glutamine or asparagine or both, with 10 ml. of 52 per cent. K_2CO_3 for varying periods. A study of these

[1] Published by permission of the Director as Contribution No. 563 of the Rhode Island Agricultural Experiment Station.

[2] Modified buffer (**2**)

7 parts 0.1 N KH_2PO_4 / 1 part 0.1 N K_2HPO_4		2.74 ml.
M/3 sodium malate		2.17 ml.
Glucose		1.00 gr.
Water	dilute to	15.0 ml.

curves indicates, as might be expected, that the break occurs when ammonia nitrogen has been completely removed, that is, after approximately two hours (curve A). From curve B, it is evident that the hydrolysis of asparagine is slight, hence the large amount of ammonia given off as shown in curve C must come from glutamine. The rate of hydrolysis of the amides at the time of complete ammonia nitrogen removal can be obtained from a consideration of the curves at the two-hour period.

Curve A	Ammonia	0.0812 mg. Ammonia–N
Curve B	Ammonia and asparagine	0.0829 mg. Ammonia–N
Curve C	Ammonia, asparagine, and glutamine	0.1012 mg. Ammonia–N

Using these figures it can be calculated from curve C that the total amount of ammonia-nitrogen evolved at the end of two hours is due to 0.0812 mg. NH_3–N, 0.0183 mg. glutamine amide nitrogen, and 0.0017 mg. asparagine amide nitrogen. The rate of glutamine hydrolysis for this time interval is approximately ten times that of asparagine.

Permutit method for ammonia determination

Sodium permutit is the usual reagent used for the estimation of ammonia. Various other permutits were prepared and studied (table I).

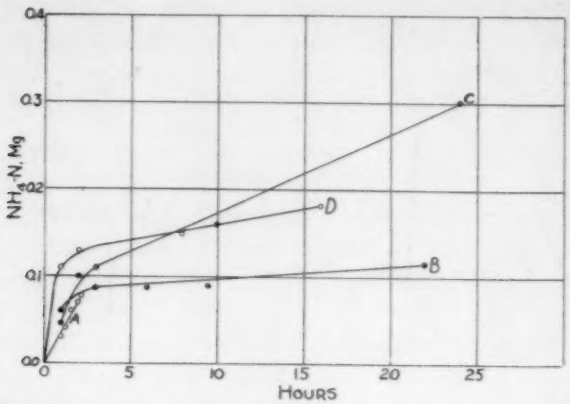

Fig. 1. Curve A, 0.0812 mg. NH_3–N.
Curve B, 0.0812 mg. NH_3–N + 1.5 mg. asparagine amide nitrogen.
Curve C, 0.0812 mg. NH_3–N + 1.5 mg. asparagine amide nitrogen + 0.959 mg. glutamine amide nitrogen.
Curve D, beet leaf juice.

These results fall in line with the lyotropic series, sodium being the ion most easily substituted, excepting lithium which was not studied. Here hydrogen permutit is somewhat out of place as it is usually the most difficult ion to displace. Sodium permutit appears to be the desirable form to use.

TABLE I

RECOVERY OF AMMONIA FROM WATER SOLUTION BY DIFFERENT PERMUTITS

PERMUTIT	PERCENTAGE RECOVERY OF 0.0812 MG. AMMONIA–N
Na	99.0
Ca	92.0
H	90.0
Mg	83.7

In view of WHITEHORN's conclusions (**6**) that permutit adsorbs amines and amino acids, a number of tests was made to determine the adsorption of glutamine and asparagine by permutit. A synthetic glutamine solution, several portions of which were adjusted to different pH values, containing 1 ml. of the previously described buffer solution and 0.959 mg. of glutamine amide nitrogen per 3 ml. were shaken with 2.5 ml. permutit for 2.5 min., the permutit was then washed and aspirated in presence of NaOH (table II).

TABLE II

NITROGEN ADSORBED FROM GLUTAMINE SOLUTIONS CONTAINING 0.959 MG. GLUTAMINE AMIDE N BY PERMUTIT CALCULATED AS PERCENTAGE OF AMIDE NITROGEN

	SOLUTION						
	1	2	3	4	5	6	7
pH of the solution	6.31	6.00	5.81	5.50	5.04	4.62	3.15
Percentage nitrogen adsorbed	1.1	0.8	1.5	0.8	1.3	1.1	1.5

Similar mixtures were prepared, using asparagine, and adsorption studies were made under conditions like those for glutamine (table III).

TABLE III

AMMONIA NITROGEN ADSORBED FROM ASPARAGINE SOLUTIONS BY PERMUTIT, CALCULATED AS PERCENTAGE OF AMIDE NITROGEN

ASPARAGINE AMIDE NITROGEN	pH 8.25	pH 7.17	pH 6.50	pH 6.44	pH 5.91	pH 5.49	pH 5.42	pH 5.15	pH 4.60	pH 3.82
mg.	%	%	%	%	%	%	%	%	%	%
0.0382	.....	.....	.....	0.79	0.79	1.31	.....	.....	0.79	0.00
0.0955	0.00	0.31	0.00	.....	.....	.....	0.52	1.04	.....	.....
0.1910	4.20	0.63	0.37	1.57	2.67	2.36	2.10	2.25	2.50	2.50
0.3820	.....	.....	.....	0.00	1.31	1.25	.....	.....	2.15	2.20
0.5730	0.98	0.98	0.26	.....	.....	.....	1.06	1.06	.....	.....
0.9050	0.39	0.38	0.34	.....	.....	.....	0.77	1.17	.....	.....
1.9100	.....	.....	.....	0.38	0.60	0.58	.....	.....	0.53	0.58

The results of a single experiment at one concentration of glutamine gives evidence of glutamine adsorption which appears, with the exception of two pH values, to be constant over the pH range used. A more detailed study of the adsorption of asparagine indicates that the least amount of adsorption takes place at pH 6.50.

The results with pure solutions show that, in the presence of these two amides, the permutit method for ammonia should give slightly high results. For example, synthetic solutions, containing varying amounts of buffer, ammonia, and asparagine were shaken with 2.5 or 0.5 ml. of permutit for 2.5 min., washed free of excess sample, and then aspirated. In most cases recoveries of ammonia somewhat higher than theoretical were obtained. Although no attempt was made to recover ammonia in the presence of glutamine it is quite obvious that, in view of the results presented in table II, such experiments would give results slightly higher than theoretical.

The low values obtained in the fifth and sixth columns, table IV, may

TABLE IV

RECOVERY OF AMMONIA IN PRESENCE OF ASPARAGINE

BUFFER*	ASPARAGINE	PERMUTIT	PERCENTAGE NH_3–N RECOVERED		
			0.0406 MG. NH_3–N ADDED	0.0812 MG. NH_3–N ADDED	0.2030 MG. NH_3–N ADDED
ml.	*mg.*	*ml.*	%	%	%
1	0.382	2.5	101	103	103
1	0.382	0.5	103	101	106
2	0.764	2.5	104	103	103
2	0.764	0.5	103	98	96
3	1.146	2.5	104	100	95
3	1.146	0.5	103	92	94

* See footnote 2, p. 701.

be explained by the fact that amounts of permutit used, 2.5 and 0.5 ml., represent a total of only 2.5[3] and 0.51 milliequivalents of exchange surface, respectively. It seems likely that with an increasing salt concentration, but with a constant amount of exchange surface, the adsorption of ammonia decreases under these conditions.

Determination of glutamine and asparagine

To determine glutamine, a juice sample obtained from leaf tissue frozen with CO_2, is hydrolyzed for two hours at pH 6.00 to 6.50, while for total amides a three-hour period in 1 N H_2SO_4 is used. Both reactions are carried out in a boiling water bath. Because of the high acid medium of the total

[3] The permutit used in this work has a total exchange capacity of 230 m.e. per 100 grams and 2.5 ml. of it weighs approximately one gram.

amide determination, it is necessary that proteins be removed from juice samples, otherwise there is a liberation of ammonia from the juice proteins during the period of hydrolysis. The conditions for glutamine hydrolysis are not sufficiently severe to cause juice protein to hydrolyze, at least with the material studied here. Table V shows both amides as determined in

TABLE V

EFFECT OF FREEZING WITH SOLID CO_2 ON GLUTAMINE AND ASPARAGINE DETERMINATIONS

FREEZING TIME	TOMATO LEAF JUICE	
	GLUTAMINE AMIDE–N	ASPARAGINE AMIDE–N
Min.	*mg./ml.*	*mg./ml.*
0	0.025	0.063
10	0.025	0.033
20	0.024	0.021
30	0.024	0.023
90	0.025	0.023
120	0.026	0.020
150	0.025	0.021

juice expressed directly from tomato leaves. The decrease in the asparagine nitrogen figure is perhaps to be attributed to the precipitation, at an extremely low temperature, of a hydrolyzable protein fraction.

Three methods of precipitating the juice protein fraction were tried: namely, heating at 70° C., Folin's sodium tungstate reagents, and 10 per cent. tannic acid. The values in table VI indicate that any one of the

TABLE VI

ASPARAGINE AND GLUTAMINE AMIDE NITROGEN IN PROTEIN-FREE JUICE*

SAMPLE	HEATED TO 70°		10 PER CENT. TANNIC ACID‡		SODIUM TUNGSTATE§	
	GLUTAMINE	ASPARAGINE	GLUTAMINE	ASPARAGINE	GLUTAMINE	ASPARAGINE
	mg./ml.	*mg./ml.*	*mg./ml.*	*mg./ml.*	*mg./ml.*	*mg./ml.*
1	0.0344	0.0080	0.0375	0.0268†	0.0325	0.0096
2	0.0367	0.0045	0.0399	0.0065	0.0340	0.0066

* Preformed ammonia was removed by shaking with permutit in all determinations.
† Incomplete precipitation of proteins.
‡ 10 per cent tannic acid, 5 ml. per 10 ml. juice.
§ Sodium tungstate 10 per cent., 1 + 1 with 0.662 N H_2SO_4, 5 ml. per 10 ml. juice.

methods, when properly used, gives results comparable to the other two; however, heat precipitation of protein is by far the most convenient. Tannic acid is satisfactory except that it contains ammonia which is imparted to the solution when the reagent is used in excess. Unless this added ammonia is removed the results for amides are higher than their true values.

The determination of ammonia in juice clarified by the three methods shown in table VI (each reagent added in excess) compared to untreated juice is as follows: 96.1 per cent., 113.6 per cent., and 98.8 per cent., respectively, showing a slight adsorbing effect of heat-coagulated protein and tungstate precipitate, as well as the influence of excess tannic acid on the ammonia content of juice after treatment. Owing to this loss of ammonia by adsorption on heat-coagulated protein it is necessary to remove the total amount of preformed ammonia nitrogen prior to the amide determination. In some instances the heat treatment is insufficient to remove all protein, for it has been observed that in the total amide determination a coagulum sometimes appears, for example with spinach juice. This can be avoided by adding one drop of 85 per cent. H_3PO_4 to the juice before heating it to 70° C.

Experiments made by adding glutamine and asparagine to tomato leaf juice show adequate recovery of these amide forms of nitrogen (table VII).

TABLE VII

RECOVERY OF GLUTAMINE AND ASPARAGINE AMIDE NITROGEN ADDED TO TOMATO LEAF JUICE

GLUTAMINE		ASPARAGINE	
ADDED	RECOVERED	ADDED	RECOVERED
mg./ml.	*mg./ml.*	*mg./ml.*	*mg./ml.*
0.0415	0.0400	0.0350	0.0380
0.0831	0.0760	0.0700	0.0720
0.1995	0.2010	0.1400	0.1290

Recommended methods of analysis

The plant sample is cut into small sections, placed in a cheesecloth bag, and frozen by a direct stream of solid CO_2 as it is formed at the end of a coil of tubing attached to a tank of liquid CO_2. After the excess CO_2 has evaporated from the frozen tissue the sample is pressed, and the juice obtained.

AMMONIA

To 2.5 ml. of sodium permutit in an ammonia aspiration tube, add an aliquot of the juice sample, (1 to 5 ml.) shake for 2.5 min. and then wash free from excess juice, thus eliminating asparagine and glutamine. The tubes are placed in the Van Slyke-Cullen aspiration block and 3 to 4 ml. water, and two drops of caprylic alcohol (an antifoam reagent) and 3 ml. 10 per cent. NaOH are added. The apparatus is connected in the usual manner and aspirated for two hours. The ammonia is caught in 0.01 N acid and determined either by Nesslerization or titration, preferably by Nesslerization.

Glutamine and asparagine

Place 10 ml. of juice in a centrifuge tube, add one drop of 85 per cent. H_3PO_4 (if necessary) heat to 70° C., cool, and centrifuge. Shake a portion of clear supernatant liquid with 2.5 ml. of permutit to remove preformed ammonia. Pipette 1 ml. of the ammonia-free juice into an ammonia tube containing 10 ml. of a phosphate buffer[4] at pH 6.40 and hydrolyze in a boiling water bath for two hours. Cool, place in aspiration block, add 2 to 3 drops of caprylic alcohol and 2 ml. of 52 per cent. K_2CO_3, aspirate two hours, and determine ammonia from glutamine by Nesslerization. Although aspiration with K_2CO_3 causes the hydrolysis of a small quantity of asparagine, the error involved is probably not much greater at this point than it would be if permutit were used to remove the hydrolyzed glutamine ammonia from the buffer mixture containing unhydrolyzed asparagine. Thus, to simplify the procedure, no attempt is made to remove the ammonia coming from glutamine by the use of permutit. One ml. of the ammonia-free sample is placed in another ammonia aspiration tube containing 1 ml. 6 N H_2SO_4 and 4 ml. water. This is hydrolyzed three hours in a boiling water bath, and cooled. Treat with sufficient 10 per cent. NaOH to make alkaline to phenolphthalein and total amide nitrogen is determined. Total amide nitrogen minus glutamine nitrogen gives asparagine amide nitrogen, on the assumption that these are the only amides present.

Discussion

The results in tables II and III showing the adsorption of small amounts of ammonia from glutamine and asparagine solutions by permutit, may be explained by the adsorption of the entire amide molecule with subsequent hydrolysis of the amide nitrogen under the alkaline conditions of the ammonia determination during aspiration, or by the adsorption of ammonia coming from the hydrolysis of the amides in solution. Experiments to test the first possibility, involving the determination of amino nitrogen in an asparagine solution before and after treatment with permutit were inconclusive because of the small amounts of amino nitrogen present.

Whitehorn (**6**) has shown that such amides as urea and its derivatives, certain amino acids, and glutamic acid hydrochloride are not adsorbed by permutit; in fact, his data indicate that only relatively strong bases are removed from solution.

Thus by accepting the indirect evidence that amide molecules are not adsorbed by permutit, the second supposition proposed above points towards the actual conditions, that is, the ammonia adsorbed by permutit when shaken with an asparagine or glutamic solution comes from the hydrolysis of the acid amide group and there is evidence to show that these amides do hydrolyze in solution.

[4] 50 ml. M/5 KH_2PO_4, 12.60 ml. M/5 NaOH, dilute to 200 ml.

If a freshly prepared glutamine solution is tested for the presence of ammonia no color appears when the Nessler reagent is added. One standing, however, even at room temperature, hydrolysis takes place and finally a strong Nessler reaction becomes evident. An asparagine solution will react in a similar manner, but more slowly, for even after two days the color reaction, though positive, is faint.

The high results for the ammonia determinations as shown in table IV, which can be considered as an error, and likewise would be expected to produce low results in the determination of the amides, may be no error at all. This can be illustrated as follows: In a plant system consisting of amides (A) and preformed ammonia (B) the conditions might be—

$$A - R\text{–}CONH_2 + 2H_2O \rightleftarrows R\text{–}COOH + NH_4OH \rightleftarrows NH_4^+ + OH^-$$

$$B - \qquad\qquad\qquad\qquad\qquad NH_4OH \rightleftarrows NH_4^+ + OH^-$$

Thus, the ammonia derived from reaction A is adsorbed by permutit (as is the free ammonia from other sources) and it can be considered accordingly, as preformed ammonia. In this system the amide nitrogen, determined by the methods presented here, would be less than that represented by the unhydrolyzed amide of A. This, however, would not necessarily be in error for, as is pointed out, this ammonia-nitrogen is considered as preformed ammonia, not as amide nitrogen. This is, of course, based on the assumption that the time of shaking plant juice with permutit, 2.5 min., is not long enough to shift the amide nitrogen-ammonia nitrogen equilibrium to the right to any great extent.

Conclusions

1. Sodium permutit under proper conditions is capable of removing quantitatively the preformed ammonia of plant juice.
2. Glutamine amide nitrogen can be determined in the presence of asparagine amide nitrogen by the use of proper conditions for hydrolysis.
3. Asparagine amide nitrogen can be estimated as the difference between total amide nitrogen and glutamine amide nitrogen.
4. Methods are proposed for these determinations.

The writer wishes to express his appreciation to Dr. H. B. Vickery for the glutamine used in this study.

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LITERATURE CITED

1. Chibnall, A. C., and Westall, R. G. The estimation of glutamine in the presence of asparagine. Biochem. Jour. **26**: 122–132. 1932.
2. Hurd-Karrer, A. Titration curves of etiolated and of green wheat seed-

lings reproduced with buffer mixtures. Plant Physiol. **5**: 307–328. 1930.

3. PUCHER, G. W. Reported at meeting of New England Section of American Society of Plant Physiologists, Amherst, Mass. May 25–26, 1934.
4. SCHLENKER, F. S. Comparison of existing methods for the determination of ammonia nitrogen and their adaptability to plant juices. Plant Physiol. **7**: 685–695. 1932.
5. VICKERY, H. B., PUCHER, G. W., CLARK, H. E., CHIBNALL, A. C., and WESTALL, R. G. The determination of glutamine in the presence of asparagine. Biochem. Jour. **29**: 2710–2720. 1935.
6. WHITEHORN, J. "Permutit" as a reagent for amines. Jour. Biol. Chem. **56**: 751–764. 1923.

SOME CHEMICAL AND RESPIRATIONAL CHANGES IN THE PAPAYA FRUIT DURING RIPENING, AND THE EFFECTS OF COLD STORAGE ON THESE CHANGES[1]

WINSTON W. JONES AND HISASHI KUBOTA

(WITH THREE FIGURES)

Papaya (*Carica papaya*) is a plant long established in Hawaii, having been brought to the Islands before 1823. It has been under study by the Hawaii Agricultural Experiment Station since 1902, but most of the investigations have been on cultural requirements and selection. The rapid rise in commercial importance of the fruit in recent years makes it necessary to know something of the changes occurring in the fruit during ripening, as a basis for harvesting, storage, and shipment.

Review of literature

THOMPSON (**13**) gives some of the early analyses of the papaya and shows that in the ripe fruit there is very little sucrose but a high percentage of reducing sugars. Her study was of several different strains, namely: Trinidad, South African, Honolulu, Barbados, Panama, and Tahiti. These strains have since lost their identity, however, and have been known at the station by their accession numbers. POPE (**6**), in a study of changes occurring during ripening, found very little sucrose in any case but a marked increase in reducing sugars during ripening. He does not state the strain with which he worked. MILLER (**4**) gives the composition and some of the uses of the papaya. WARDLAW (**14**) has shown that at the beginning of ripening (*i.e.*, the beginning of the development of the yellow color) there is a marked increase in the production of carbon dioxide, which reaches a high point (climacteric peak) at the time of full ripeness. After this peak, there is a decrease in carbon dioxide evolution. He further shows that the carbon dioxide content of the fruit cavity increases with maturity. WILCOX (**15**) found that the papaya can be held in cold storage at 32° to 34° F. (0 to 1.5° C.) for 45 days, but the fruit is subject to decay. JONES (**1**) has shown that papayas can be held at 32° to 34° F. for at least 12 days if the fruit is ripe when placed in storage. The ripening process is apparently permanently stopped in unripe fruit held at this temperature.

Materials and methods

The fruits used in this investigation were all of the type IV (**10, 11**) Solo strain, and were grown in the Station's experimental plots at Kailua, Oahu.

[1] Published with the approval of the Director, Hawaii Agricultural Experiment Station as technical paper no. 57.

The fruits were harvested as needed. Owing to the nature of growth of the papaya, it is difficult to obtain a representative composite sample. In a few cases, a sample of three fruits was used but in most cases means of analyses of similar individual fruits were used.

Methods of analysis were standard: Sugars were determined by the method of STILES, PETERSON, and FRED (9); nitrogen, by the reduced iron method of PUCHER, LEAVENWORTH, and VICKERY (8), adapted to the Micro-Kjeldahl method described by PREGL (7); and carbon dioxide by the method of THOMAS (12) and MITCHELL (5).

Presentation of data

NORMAL RIPENING PROCESS

From the time of the beginning of ripening in the fruit until full ripeness is attained, the process is continuous. The first noticeable change is the development of yellow color in the funiculus. The color then spreads outward, and full ripeness is attained when the outer surface is completely yellow. Accompanying the change in color, the physical characteristics of the flesh change from very tough and rubbery to very soft and crushable.

The data presented in table I show the differences in concentration of sugars and other constituents of papaya harvested at three stages of maturity.

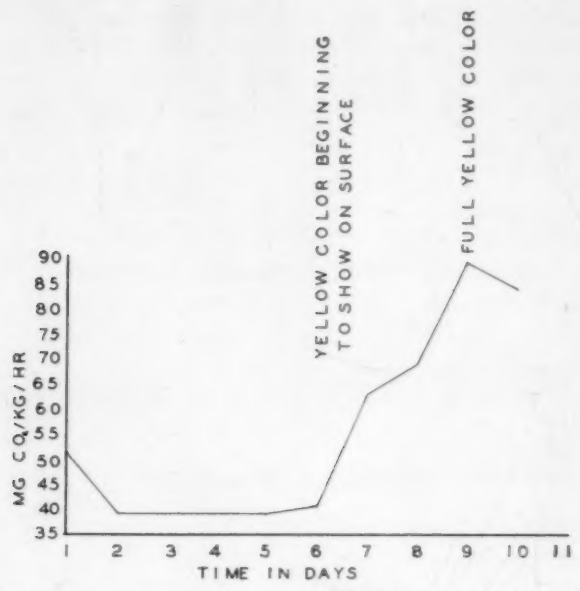

FIG. 1. Respiration of papaya fruit during ripening at approximately 25° C.

Figure 1 shows the carbon dioxide given off by fruit harvested mature-green and allowed to ripen at room temperature. Figure 2 shows the percentage of carbon dioxide and oxygen contained in the cavities of fruits harvested at different stages of maturity.

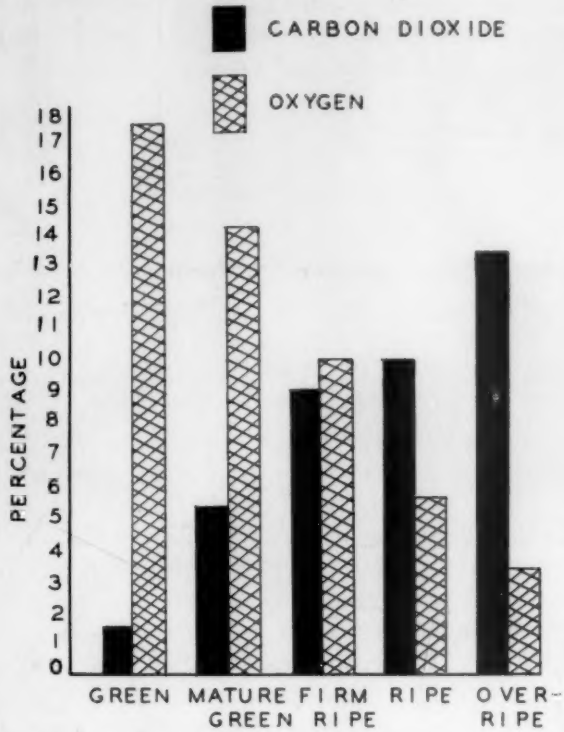

FIG. 2. Carbon dioxide and oxygen content of the gas in the internal cavity of the papaya at different stages of maturity.

CHEMICAL CHANGES OCCURRING DURING RIPENING

From table I it may be seen that during the ripening process a change occurs in the relationship between sucrose and reducing sugars. In the green fruit, 42 per cent. of the total sugar occurs as sucrose, while in the ripe fruit only 18 per cent. of the total sugar occurs as sucrose. The acid-hydrolyzable material drops from 9.33 per cent. of the total dry weight in the green fruit to 4.36 per cent. in the ripe fruit. As there is no starch present in the fruit, this decrease represents a change in cell-wall constituents, and accounts, at least in part, for the change in consistency of the flesh during ripening. There is apparently no significant change in the nitrogen content during ripening.

TABLE I

COMPOSITION* OF PAPAYA FRUIT AT DIFFERENT STAGES OF MATURITY

MATURITY	H_2O	SOLUBLE SOLIDS	REDUCING SUGAR	SUCROSE	TOTAL SUGAR	SOLUBLE NITROGEN	INSOLUBLE NITROGEN	TOTAL NITROGEN	ACID-HYDROLYZABLE MATERIAL
	%	%	%	%	%	%	%	%	%
Ripe	85.3	11.56	62.64	14.40	79.04	0.28	0.25	0.53	4.36
Firm ripe	86.1	10.67	62.23	12.41	74.64	0.35	0.31	0.66	4.74
Mature-green	86.1	10.49	40.70	30.98	71.68	0.29	0.30	0.59	9.33

* Water and soluble solids as percentage of fresh weight; others as percentage of dry weight.

RESPIRATIONAL CHANGES OCCURRING DURING RIPENING

Carbon dioxide elimination and the carbon dioxide and oxygen content of the internal cavity were followed from the beginning of ripening to overripeness. The carbon dioxide elimination was measured on fruit harvested mature but green and allowed to ripen, while the internal gases were measured in fruit harvested at progressive stages of ripeness. Figure 1 shows the carbon dioxide elimination of approximately one dozen individual fruits measured separately. It will be noted that in the firm-ripe fruit (*i.e.*, with the yellow color just beginning to show) the rate of carbon dioxide elimination is represented by a low curve parallel with the base line. As the color develops, the rate of respiration, as measured by carbon dioxide elimination, rapidly increases and reaches a peak simultaneously with full color development. After this peak, the rate of respiration decreases. In some instances it increases again but this can be attributed to the growth of fungi on the overripe fruit and to the leakage of internal gases through the wounds caused by the fungi. Figure 2 shows that long before the development of external color and before the rate of external elimination of carbon dioxide begins to increase, the concentration of carbon dioxide in the internal atmosphere has begun. This increase in carbon dioxide in the internal atmosphere continues throughout the ripening period and past full ripeness. Thus, there is no peak of internal carbon dioxide concentration. As the carbon dioxide content of the internal cavity increases, the oxygen content decreases.

EFFECT OF COLD STORAGE ON THE OBSERVED RIPENING PROCESS

Fruit at three stages of ripeness—mature but green; firm ripe with yellow color just beginning to show; and ripe, with full yellow color but firm—were stored at 3° to 4° C. for 11 days. There were no indications of ripening during the period, and at the end of the cold storage treatment

there were no noticeable changes in the fruit. On removal to room temperature (approximately 25° C.) the green fruit did not ripen, but small sunken spots developed and in the course of 2 or 3 days the fruits were overrun with fungi. The firm-ripe fruit reacted very much as the green, never ripening normally. The ripe fruits were in essentially the same condition after cold storage as before but were subject to rapid breakdown and fungal attack when held at room temperature.

Effect of cold storage on the chemical changes occurring during ripening

Table II shows the composition of mature-green fruit before and after cold storage, and when allowed to ripen at room temperature without any

TABLE II

Composition* of papaya fruits when harvested mature-green, when harvested mature-green and allowed to ripen at room temperature, and when harvested mature-green and held for 11 days at 3°–4° C.

Maturity	H_2O	Soluble solids	Reducing sugar	Sucrose	Total sugar	Soluble nitrogen	Insoluble nitrogen	Total nitrogen
	%	%	%	%	%	%	%	%
Mature-green	86.1	10.49	40.70	30.98	71.68	0.29	0.30	0.59
Harvested mature-green; ripe after 5 days at room temperature	86.9	9.96	56.22	8.00	64.22	0.44	0.34	0.78
Mature-green —11 days at 3°–4° C.	86.9	9.40	29.24	33.02	62.26	0.35	0.39	0.74

* Water and soluble solids as percentage of fresh weight; others as percentage of dry weight.

cold storage. It will be noted that, during 11 days at 3° to 4° C., there was no such hydrolysis of sucrose as occurred when fruit was allowed to ripen at room temperature. The quantitative differences in sugar content among the individual fruits are probably due to variability and should not be considered when comparing the treatments. The important point seems to be the relation between reducing sugars and sucrose.

Effect of cold storage on respirational changes

Figure 3 shows the carbon dioxide elimination of firm-ripe fruit stored at 3° to 4° C. during a period of 11 days. It will be noted that the rate of elimination changes very little over the 11-day period. It will also be noted that there is no climacteric rise in respiration during cold storage.

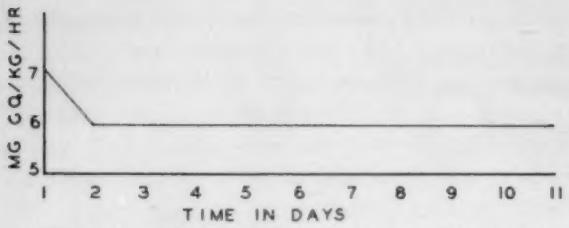

FIG. 3. Respiration of papaya fruits at 3° to 4° C.

Discussion

Ripening of the papaya, once begun, proceeds rapidly at room temperature. Within 3 or 4 days after the appearance of the first yellow color, the fruit is fully ripe. During this short period, several important changes occur: A large percentage of the sucrose is hydrolyzed, a climacteric rise in carbon dioxide elimination occurs, the flesh of the fruit changes from a very tough and rubbery to a soft, easily crushed texture, and the color changes from green to yellow. Unlike some other fruits, such as apples, the climacteric rise occurs in the papaya simultaneously with ripening and not before ripening.

Since the modification of the quarantine law to allow the entrance of Hawaiian fruits into the mainland after a cold treatment, the effects of cold storage on the papaya become of especial interest. The results presented show that cold storage halts the ripening processes in the papaya permanently rather than just for the duration of storage, as is the case with pears (**2, 3**). On removal of the papaya from cold storage (11 days at 1.7° C.) to room temperature, small sunken dead spots develop in the course of 1 or 2 days. These dead spots serve as places of entrance for fungi; within 3 to 4 days the fruit is overrun with the fungi, and breakdown proceeds without ripening. These results agree with those reported by WARDLAW (**14**).

Summary

1. The normal process of ripening in the papaya is described and the effect of cold storage on this process discussed.

2. During the ripening process a large portion of the sucrose is hydrolyzed, the color changes from green to yellow, the flesh softens, and there is a rise in carbon dioxide elimination.

3. Cold storage stops the ripening process and causes a chill effect so that on removal to room temperature the ripening process is not resumed.

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LITERATURE CITED

1. JONES, WINSTON W. Papaya storage. Hawaii Agr. Exp. Sta. Ann. Rept. 1937, pp. 28–30. 1937.

2. MAGNESS, J. R. Investigations in the ripening and storage of Bartlett pears. Jour. Agr. Res. **19**: 473–500. 1920.
3. MARTIN, WILLIAM E. Chemical study of the ripening process of Bosc pears. Bot. Gaz. **99**: 42–68. 1937.
4. MILLER, CAREY D., BAZORE, K., and ROBBINS, R. C. Some fruits of Hawaii—their composition, nutritive value and use. Hawaii Agr. Exp. Sta. Bull. 77. 1936.
5. MITCHELL, JOHN W. A method of measuring respiration and carbon fixation under controlled environmental conditions. Bot. Gaz. **97**: 376–387. 1935.
6. POPE, W. T. Papaya culture in Hawaii. Hawaii Agr. Exp. Sta. Bull. 61. 1930.
7. PREGL, FRITZ. Quantitative organic microanalysis. P. Blakiston's Son and Co., Inc., Phila.
8. PUCHER, G. W., LEAVENWORTH, C. S., and VICKERY, H. B. Determination of total nitrogen of plant extracts in presence of nitrates. Ind. & Eng. Chem. Anal. Ed. 2: 191–193. 1930.
9. STILES, H. R., PETERSON, W. H., and FRED, E. B. A rapid method for the determination of sugar in bacterial cultures. Jour. Bact. **12**: 427–439. 1926.
10. STOREY, W. B. The primary flower types of Papaya and the fruit types that develop from them. Proc. Amer. Soc. Hort. Sci. **35**: 80–82. 1938.
11. ————. Segregations of sex types in Solo Papaya and their application to the selection of seed. Proc. Amer. Soc. Hort. Sci. **35**: 83–85. 1938.
12. THOMAS, M. D. Precise automatic apparatus for continuous determination of carbon dioxide in air. Ind. & Eng. Chem. Anal. Ed. **5**: 193–198. 1933.
13. THOMPSON, A. The composition of Hawaiian fruits and nuts. Hawaii Agr. Exp. Sta. Rept. 1914: 62–75. 1915.
14. WARDLAW, C. W., and LEONARD, E. R. The storage and physiology of tropical fruits. Trop. Agr. **12**: 313–319. 1935.
15. WILCOX, E. V., and HUNN, C. J. Cold storage for tropical fruits. Hawaii Agr. Exp. Sta. Press Bull. 47. 1914.

A SEMI-MICRO METHOD FOR THE DETERMINATION OF REDUCING SUGARS

SAM G. WILDMAN AND ELMER HANSEN

(WITH THREE FIGURES)

Introduction

The MUNSON-WALKER method (3) with the modification of BERTRAND (1) has long been used for the determination of reducing sugars in plant extracts. The large amounts of solutions required by this method, however, make necessary the frequent preparation and standardization of solutions, which is inconvenient when large numbers of determinations are being made over an extended period of time. For plant materials containing not more than 10 to 12 per cent. sugar, it seems unnecessary to use the amounts of Fehling's solutions originally specified. ILJIN (2) in adapting the MUNSON-WALKER method to a micro scale, used 3-ml. portions of Fehling's solutions A and B. Where more than one per cent. is encountered, however, the dilutions made necessary by this modification in order to keep within his range of 1 to 25 mg. of sugar, result in too great a dilution factor in the final value. An additional advantage of reducing the amounts of reagents used would be in making possible carrying out the reduction and subsequent washing of the copper oxide precipitate directly in centrifuge tubes, thus eliminating the tedious and inconvenient procedure of filtering the precipitate on a Gooch crucible. The use of the centrifuge for micro-sugar analysis has been reported by ILJIN (2) and PHILLIPS (4).

Another objection to the MUNSON-WALKER method is the practice of boiling the sugar solutions over an open flame. QUISUMBING and THOMAS (5) have shown that this practice is subject to error, since at 100° C. the end-point of the copper reduction is taken on a continuously ascending curve, so that small changes in time or temperature will result in appreciable error. They pointed out the desirability of using a temperature at which the reduction curve would eventually become level, at which point the end-point could be taken. A temperature of 80° C. was found best for this purpose. SHAFFER and SOMOGYI (8) have also stressed the importance of taking the end-point of reduction on the level portion of the curve.

The use of standard potassium permanganate solution for titrating the reduced cuprous oxide according to the BERTRAND modification, has the disadvantages of instability of solution, sensitiveness to foreign organic matter and poor end-point, especially when the solutions cannot be thoroughly cleared. In view of the recent development of an inside indicator for potassium dichromate (7), it would appear desirable to use this solution instead of potassium permanganate for titration of the reduced copper oxide.

With the above considerations in mind, the MUNSON-WALKER-BERTRAND method has been modified to a semi-micro scale by incorporating the following changes:

(1) Using 20 ml. of Fehling's solution instead of 50 ml.
(2) Carrying out the sugar-copper reactions in an 80° C. water bath instead of boiling over an open flame.
(3) Centrifuging the Cu_2O precipitate instead of filtering on a Gooch crucible.
(4) Using potassium dichromate with sodium diphenylamine sulphonate inside indicator as a standard oxidant instead of potassium permanganate.

Preliminary experimentation

PREPARATION OF REAGENTS.—Fehling's solutions A and B were prepared according to the modification proposed by QUISUMBING and THOMAS (5) as follows:

Solution A was prepared by dissolving 69.28 gm. of c.p. $CuSO_4 \cdot 5H_2O$ in warm water and then diluting to 1 liter in a volumetric flask. After standing for several hours, the solution was filtered and transferred to a Pyrex reagent bottle.

Solution B was prepared by dissolving 346 gm. of c.p. sodium potassium tartrate in warm water and then transferring to a 1-liter volumetric flask. A saturated solution of sodium hydroxide was then digested on a steam bath until insoluble carbonates had settled out. The exact alkalinity of the solution was then determined and the amount containing exactly 130 gm. of sodium hydroxide added to the sodium potassium tartrate solution and made to 1 liter.

Ferric ammonium sulphate solution was made by dissolving 240.9 gm. of $Fe(NH_4)(SO_4)_2 \cdot 12\ H_2O$ in warm water, then adding 200 ml. of concentrated H_2SO_4. After cooling, the solution was diluted to 1 liter and filtered.

A 0.1000-N solution of potassium dichromate was prepared by dissolving 4.9037 gm. of highest purity $K_2Cr_2O_7$ (previously dried) in distilled water and then diluting to 1 liter in a volumetric flask.

Sodium diphenylamine sulphonate indicator was made by dissolving 0.32 gm. of barium diphenylamine sulphonate in 100 ml. of water, then adding 1.0 gm. of sodium sulphate and allowing the precipitate of barium sulphate to digest for one hour. The solution of sodium diphenylamine sulphonate was then filtered through a heavy filter paper.

DETERMINATION OF SUGAR-COPPER REDUCTION TIME.—QUISUMBING and THOMAS (5) have shown that 100 ml. of sugar-copper solutions held in an 80° C. water bath required 30 min. for complete reduction. Since smaller quantities of solutions were to be used, the possibility of reducing the time for reduction established by these investigators was investigated. Three

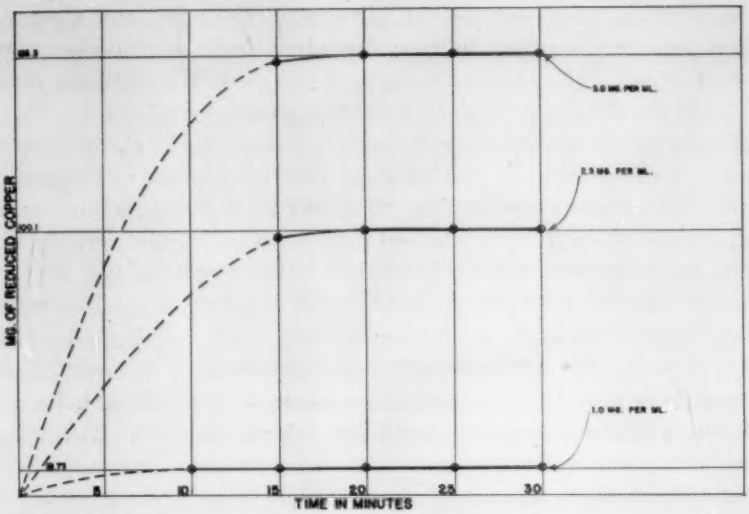

FIG. 1. Time required for digestion of varying concentrations of glucose and an alkaline copper tartrate solution in an 80° C. water bath.

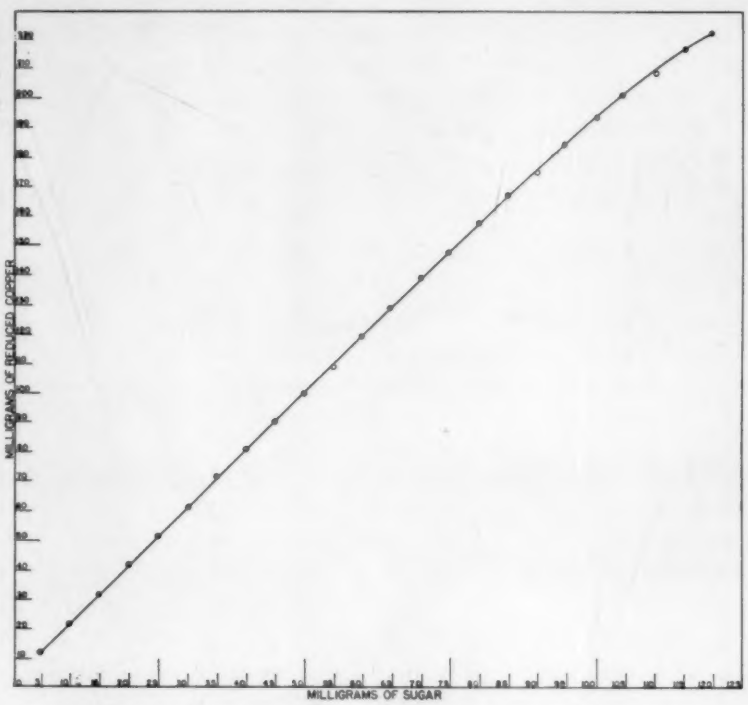

FIG. 2. Copper-glucose curve, determined by experiment.

glucose solutions were prepared containing 1 mg., 2.5 mg., and 5 mg. respectively per ml., representing the maximum, minimum, and median range of the sugar curve. After mixing with 20 ml. of Fehling's solutions, duplicate samples of the three sets were subjected to time intervals of 10, 15, 20, 25 and 30 minutes in an 80° C. water bath regulated to 1° C. by two bunsen burners. In this case, the results show (fig. 1) that the reduction curves became level shortly after 15 min., remained so at 20 to 25 min., and only slightly ascended in 30 min. In order to preserve uniformity, the time of 20 min. was arbitrarily accepted for further experimentation.

ARRANGEMENT OF THE CENTRIFUGE.—For these investigations an International type 1C centrifuge with an 8-place head carrying 50 ml. Pyrex tubes was used. To facilitate the rapid balancing of the centrifuge, the metal carriers were weighed and balanced to within 0.05 gm. by placing small pieces of sheet copper beneath the rubber cushions. The weight of the heaviest glass centrifuge tube was then determined, and the deficiencies in weight of the other seven, varying from 0.20 to 2.40 gm., recorded on the

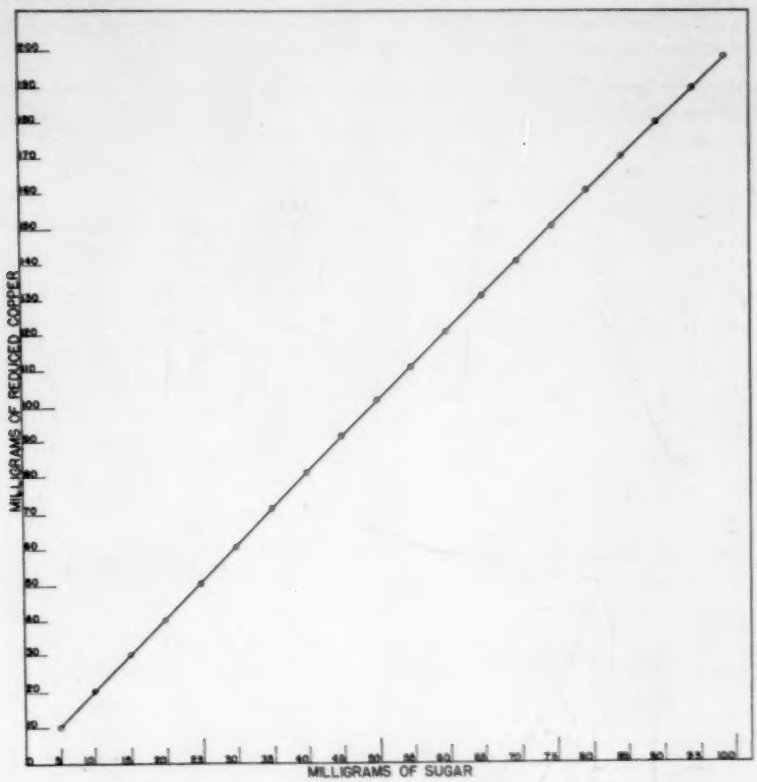

FIG. 3. Copper-sucrose curve, determined by experiment.

tubes with a glass-marking pencil. Hence, the centrifuge was rapidly balanced by the simple expedient of adding the same amount of water (measured from a burette set up for the purpose) in milliliters as recorded in grams on the centrifuge tubes. Since the centrifuge was designed to accommodate eight tubes at one time, two sets of eight tubes were prepared.

Method of analysis

As a result of preliminary experimentation, the following analytical procedure was adopted. To 20 ml. of sugar solution contained in a 50-ml. Pyrex centrifuge tube, measure from a burette 10 ml. each of Fehling's solutions A and B. Place in a water bath held at 80° C. (± 1° C.) for 20 min. While the first set of eight tubes is digesting, another set may be prepared to be placed in the water bath as soon as the first set is removed for centrifuging, etc. If this procedure is followed, 16 determinations may be made

TABLE I

GLUCOSE* $y = 0.4650x + 0.00028x^2$

COPPER	SUGAR	0.1000N $K_2Cr_2O_7$	COPPER	SUGAR	0.1000N $K_2Cr_2O_7$	COPPER	SUGAR	0.1000N $K_2Cr_2O_7$
mg.	*mg.*	*ml.*	*ml.*	*mg.*	*ml.*	*mg.*	*mg.*	*ml.*
5.0	2.33	0.78	85.0	41.55	13.31	165.0	84.37	25.85
10.0	4.67	1.57	90.0	44.12	14.10	170.0	87.04	26.68
15.0	7.04	2.38	95.0	46.71	14.90	175.0	89.94	27.25
20.0	9.41	3.14	100.0	49.30	15.70	180.0	92.78	28.21
25.0	11.80	3.93	105.0	51.92	16.49	185.0	95.65	29.00
30.0	14.20	4.71	110.0	54.55	17.25	190.0	98.48	29.80
35.0	16.62	5.50	115.0	57.18	18.05	193.3†	100.0	30.40
40.0	19.06	6.28	120.0	59.84	18.84	199.6	105.0	31.42
45.0	21.49	7.07	125.0	62.51	19.60	208.5	110.0	32.80
50.0	23.96	7.85	130.0	65.18	20.38	216.2	115.0	34.00
55.0	26.43	8.62	135.0	67.87	21.19	222.5	120.0	35.0
60.0	28.91	9.40	140.0	70.59	21.98			
65.0	31.41	10.20	145.0	73.33	22.75			
70.0	33.93	10.80	150.0	76.06	23.50			
75.0	36.46	11.74	155.0	78.80	24.25			
80.0	38.99	12.51	160.0	81.57	25.08			

* Sugar values for both glucose and sucrose were calculated from experimentally determined data (see fig. 2 and fig. 3) by the method of least squares according to the equations:

(1) $y = a + bx + ex^2$

(2) $b = \dfrac{\Sigma(x^4)\Sigma(xy) - \Sigma(x^2)\Sigma(x^2y)}{\Sigma(x^2)\Sigma(x^4) - [\Sigma(x^3)]^2}$

(3) $c = \dfrac{\Sigma(x^2)\Sigma(x^2y) - \Sigma(x^3)\Sigma(xy)}{\Sigma(x^2)\Sigma(x^4) - [\Sigma(x^3)]^2}$

The mean eror, calculated from the equation $d = \sqrt{\dfrac{\Sigma d^2}{n}}$ is 0.251 mg. for glucose and 0.228 mg. for sucrose.

† Values determined experimentally.

in approximately one hour. Remove the tubes and cool for 2 to 3 minutes in cold water. Equalize the weight of the tubes by adding water to each in accordance with its deficit in weight, and centrifuge at not less than 1000 r.p.m. for 4 min. If some light flakes of Cu_2O persist in floating after centrifuging, add 2 to 4 drops of n-butyl alcohol to reduce the surface tension. Decant the excess Fehling's solution from the Cu_2O precipitate with a stream of water from a wash bottle, add 5 to 10 ml. of ferric ammonium sulphate solution, depending upon the amount of cuprous oxide present. Transfer the solution quantitatively to a 150-ml. beaker, add 6 to 9 drops of sodium diphenylamine indicator and titrate with standard potassium dichromate solution. At the end point, the color of the solution changes from an emerald green to an intense purple, and in practice it is not difficult to detect this change with ½ drop of potassium dichromate. Duplicate titrations should check within 0.05 ml.

Determination of sugar-copper ratios

Using the procedure outlined, the ratios of copper to glucose and invert sugar were determined. For this purpose stock solutions containing 5 mg. of sugar per milliliter were prepared from Pfanstiehl's highest purity glucose and sucrose, dried for 2 hours in a vacuum oven at 70° C. previous to weighing. Stock solutions of sucrose were hydrolyzed at pH 5.0 with 2 ml. of Difco invertase solution for 4 hours. The experimental curves showing the copper equivalents to glucose and invert sugar and glucose are shown

TABLE II

INVERT SUGAR* $y = 0.4594x + 0.00028x^2$

COPPER	SUGAR	0.1000N $K_2Cr_2O_7$	COPPER	SUGAR	0.1000N $K_2Cr_2O_7$	COPPER	SUGAR	0.1000N $K_2Cr_2O_7$
mg.	*mg.*	*ml.*	*mg.*	*mg.*	*ml.*	*mg.*	*mg.*	*ml.*
5.0	2.30	0.77	85.0	41.06	13.31	165.0	83.43	25.85
10.0	4.66	1.57	90.0	43.61	14.10	170.0	86.18	26.68
15.0	6.90	2.38	95.0	46.17	14.90	175.0	88.96	27.25
20.0	9.30	3.14	100.0	48.74	15.70	180.0	91.76	28.21
25.0	11.64	3.93	105.0	51.32	16.49	185.0	94.61	29.00
30.0	14.03	4.71	110.0	53.93	17.25	190.0	97.41	29.80
35.0	16.42	5.50	115.0	56.53	18.05	195.0	101.2	30.62
40.0	18.83	6.28	120.0	59.16	18.84	200.0	113.1	31.42
45.0	20.24	7.07	125.0	61.80	19.60			
50.0	23.67	7.85	130.0	64.44	20.38			
55.0	26.11	8.62	135.0	67.11	21.19			
60.0	28.58	9.40	140.0	69.80	21.98			
65.0	31.04	10.20	145.0	72.61	22.75			
70.0	33.53	10.80	150.0	75.22	23.50			
75.0	36.03	11.74	155.0	77.93	24.25			
80.0	38.56	12.51	160.0	80.67	25.08			

* See footnote at the bottom of table I.

in figures 2 and 3. The sugar values for both invert sugar and glucose were calculated from the experimental data by the method of least squares and are shown in tables I and II.

Summary

1. A semi-micro modification of the MUNSON-WALKER-BERTRAND method for determining reducing sugars has been presented.

2. The method described uses smaller quantities of reagents, substitutes a water bath for digestion, centrifuges the cuprous oxide precipitate, and uses potassium dichromate as a standard oxidant.

The authors wish to acknowledge their indebtedness to Dr. W. M. ATWOOD for his helpful suggestions and for the use of his laboratory, and to Dr. W. E. MILNE for his suggestions on the calculation of the equations for the sugar curves.

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LITERATURE CITED

1. BERTRAND, G. Le dosage des sucres reducteurs. Bull. Soc. Chim., ser. 3, **35**: 1285–1299. 1906.
2. ILJIN, W. S. Bestimmung des Zuckers mittels Fehlingscher Lösung und Zentrifugierens. Biochem. Zeit. **193**: 322–325. 1928.
3. MUNSON, L. S., and THOMAS, P. H. The unification of reducing sugar methods. Jour. Amer. Chem. Soc. **28**: 663–686. 1906.
4. PHILLIPS, T. G. The determination of sugars in plant extracts. Jour. Biol. Chem. **95**: 735–742. 1932.
5. QUISUMBING, F. A., and THOMAS, A. W. Conditions affecting the quantitative determination of reducing sugars by Fehling solution. Elimination of certain errors involved in current methods. Jour. Amer. Chem. Soc. **43**: 1503–1526. 1921.
6. WILLARD, H. H., and FURMAN, N. H. Elementary Quantitative Analysis. 1933.
7. SHAFFER, P. A., and SOMOGYI, M. Copper-iodometric reagents for sugar determinations. Jour. Biol. Chem. **100**: 695–713. 1933.

SAND CULTURE STUDIES OF THE USE OF SALINE AND ALKALINE WATERS IN GREENHOUSES

V. G. Heller, R. H. Hageman and E. L. Hartman

(WITH FOUR FIGURES)

Introduction

Saline and alkaline waters, which are widely distributed over the earth's surface and are frequently found in the southwestern portion of the United States, are responsible for many troubles encountered in greenhouse production. The contamination comes from deep wells, salt beds, gypsum deposits, and alkaline planes, and is primarily composed of sodium, calcium, and magnesium chlorides, sulphates, or bicarbonates. Frequently the concentration of these waters approaches that of sea water. The use of such waters for plants is often disastrous.

The action of these salts occurring singly or in combination has frequently been discussed in connection with field irrigation. Breazeale of Arizona, Lipman of California, and Harris of Utah and their associates are especially deserving of credit for their investigations. Results of these field trials, however, cannot be safely applied to greenhouse problems, for here we find more frequent applications of water, more rapid evaporation, shallow beds, and a continuous concentrated production.

The effect and toxic levels of the various alkaline salts on greenhouse plants, as shown by accumulation of the salts in the soil has been reported by this station (**6**). The purpose of this experiment was to make a similar study of the effect of sodium chloride and sodium bicarbonate without accumulation of the salt in the soil solution.

Experimental

Comparable four-inch plants of Marglobe tomatoes were transferred to pots filled with silica and watered with a nutrient solution consisting of:

139.5 ml. of 0.5 mol. $MgSO_4$
139.5 " " " " KH_2PO_4
174.4 " " " " $CaNO_3$
15.2 " iron citrate soln. (4 gm. per 100 ml.)
15.2 " A–Z soln. as suggested by Haas (**5**)

This mixture was diluted to four gallons with rain water and the desired kind and quantity of salt was added to make the respective solutions. pH determinations were made on each solution after the salts were added. The plants were watered frequently enough to provide for water requirements. Every seven days all pots were leached to prevent accumulation, and to

maintain a nearly constant concentration of salt. (Analysis of the sands at the close of the experiment proved little or no accumulation had occurred). Immediately after leaching, the plants were again watered with their respective solutions.

Daily records were made of the amount of solution used for each lot of five, the general appearance as to growth, the quantity and color of foliage, the length of life, and, finally, the average dry weight. At the completion of the tests, all plants were clipped at the ground level, dried, ground, and analyzed according to the methods of the Association of Official Agricultural Chemists. The results from the tomatoes receiving sodium chloride and sodium bicarbonate solutions varying from 100 to 3000 parts per million in concentration are presented in the accompanying figures.

Results and discussion

The plants in both series were uniform in appearance and in excellent condition at the start. Growth was rapid during the first week and no injuries were observed. Thereafter, injuries in the form of stunted growth and yellowed and dead leaves were noted in the higher concentrations. These injuries moved progressively to the lower salt concentrations as the experiment was continued. No sharp demarcation of injury could be detected, but a gradual increase with increased salt concentration was noticeable.

Injury was not as characteristic, and appeared more slowly, in the sodium chloride series than in the sodium bicarbonate. Chlorotic leaves appeared at the end of the third week on plants receiving concentrations of sodium chloride greater than 2500 parts per million. At the termination of the experiment, all plants treated with more than 1500 parts per million sodium chloride developed turgid, yellowish leaves which curled and then died. These conditions were very pronounced in the higher concentrations, 2300 to 3000 parts per million. BASLAVSKAJA (**1**) observed similar conditions in the leaves of potato plants treated with chloride salts and attributed them to the chloride ion. Plants receiving over 2000 parts per million of salt in both series were much more resistant to wilting, and their total water consumption was reduced one-third or more for the duration of the experiment.

When the experiment was terminated, observations indicated that salt injuries were apparent in concentrations as low as 900 parts per million of sodium bicarbonate and 1100 parts per million of sodium chloride. General condition of the plants indicated that concentrations greater than 1600 parts per million of sodium bicarbonate and 1700 parts per million of sodium chloride were toxic.

Data for the total growth of plants, represented by wet and dry weights of each group of five plants, are given in figures 1 and 2 for the sodium chloride and sodium bicarbonate series. In the sodium chloride series, the

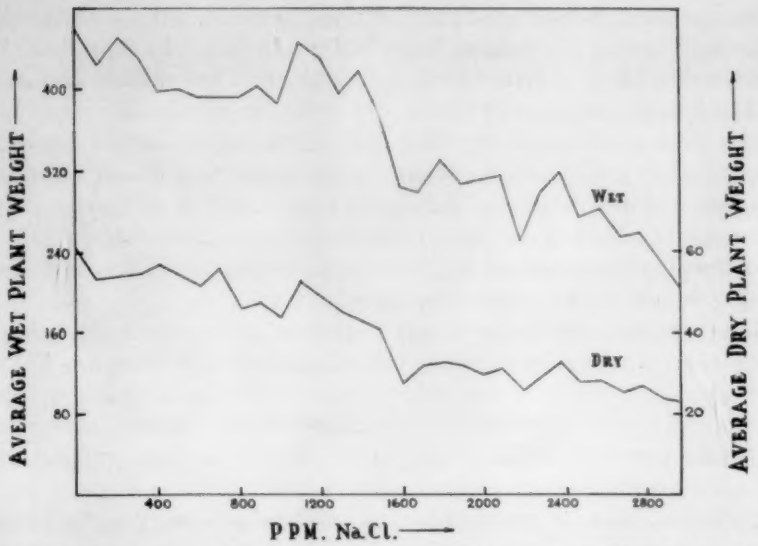

FIG. 1. Growth curves for tomato plants in sand cultures with NaCl in the nutrient solutions. Upper curve constructed on wet weights; lower curve on dry weights.

green weight of plants receiving over 2900 parts per million of the salt was one-half that of the checks. A similar retardation of growth as shown by its dry weight occurred at a concentration of 1600 parts per million sodium chloride. Since the plants which received over 2000 parts per million salt

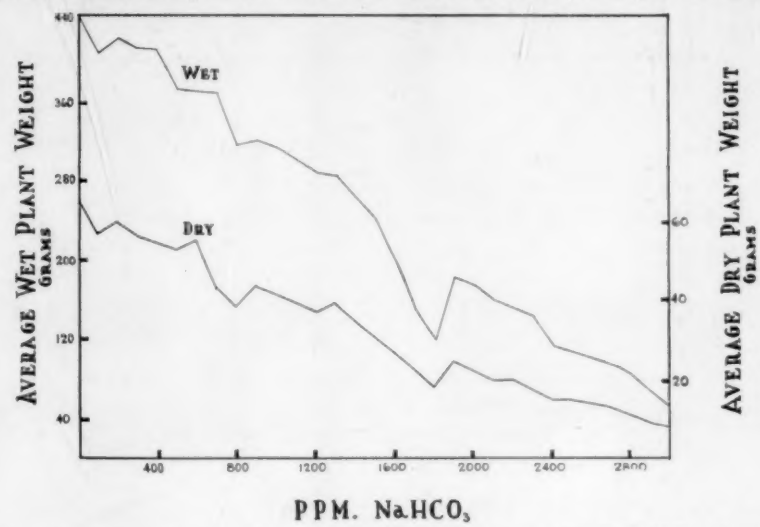

FIG. 2. Growth curves of tomato plants in sand culture with $NaHCO_3$ in the culture solutions.

had an increased moisture content of 3 to 3.5 per cent. more than the checks, the injury based on dry weights is the better criterion. In the sodium bicarbonate series, plants watered with over 1600 parts per million had a green weight one-half that of the checks. On a similar comparative basis of dry weights the concentration was 1400 parts per million. In the latter series the increase in the moisture content of the plants was not as pronounced. The green and dry weights of the checks were larger in all cases than those of the plants receiving the salt water treatment. BREAZEALE's (2) work with wheat seedlings treated with sodium chloride shows this same gradual declination in growth as concentration is increased.

Observations failed to detect any reduction in growth in the lower concentrations. The groups receiving salt concentrations lower than 600 parts per million appeared to be equivalent to or better than the checks. This is not in accord with LIPMAN's (7) statement that small concentrations of about 500 to 1000 parts per million sodium chloride may depress growth in early stages, but that higher concentrations may stimulate it.

Analysis of data presented in figures 3 and 4 shows many marked changes

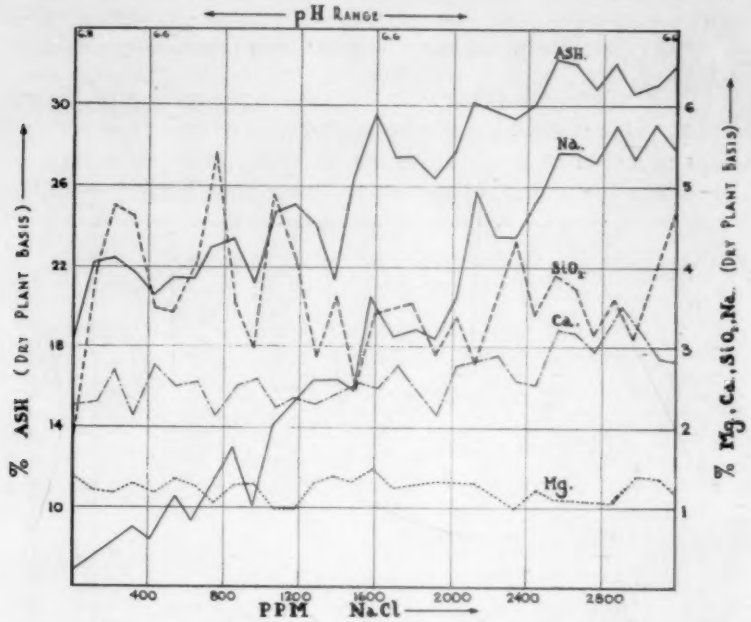

FIG. 3. Ash content of tomato plants grown in sand cultures with additions of NaCl in the nutrient solutions. Dry weight basis.

in the plants as they were subjected to increased concentrations of salts.

The ash content of plants treated with sodium chloride increased very rapidly and reached values as high as 32 per cent. of the dry plant weight.

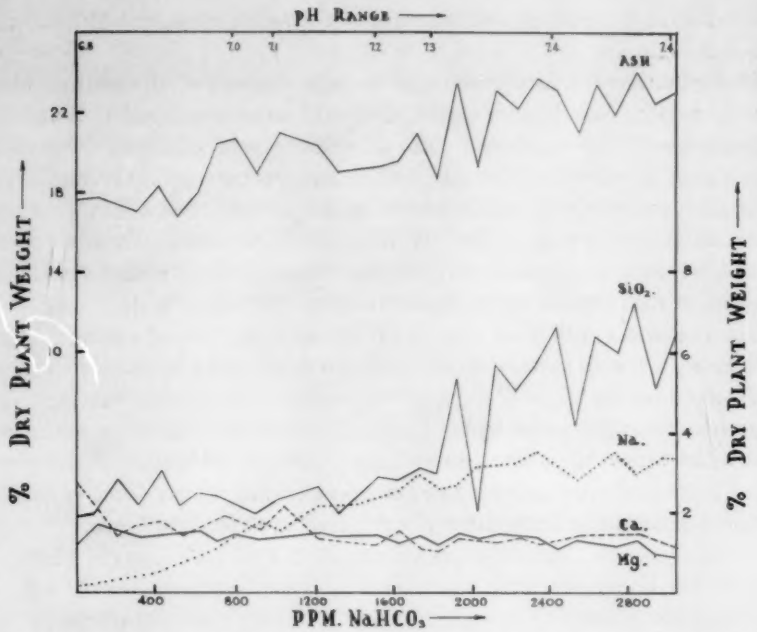

FIG. 4. Ash content of tomato plants grown in sand cultures with additions of $NaHCO_3$ to the nutrient solutions. Dry weight basis.

The addition of 100 parts per million sodium chloride to the solutions increased the ash content 4 per cent. of dry plant weight over the checks, and 1500 parts per million sodium chloride gave an increase of 11 per cent. Plants treated with concentrations of sodium chloride between 1300 and 1500 parts per million showed the most rapid increase in the ash content. The ash content of the sodium bicarbonate series did not increase as rapidly as that of the sodium chloride series, and did not reach exceedingly high values. Plants receiving from 500 to 800 parts per million sodium bicarbonate had a rather marked increase in ash.

The sodium content of plants treated with the higher concentrations of sodium chloride was over twenty times that of the checks. Plants receiving solutions containing between 1800 and 2000 parts per million of sodium chloride showed a very marked increase of sodium from 2.5 to nearly 5.0 per cent. of the dry plant weight. Although determination of the chloride ion was not made, it would be logical to assume that the sodium chloride content was increased. BUFFUM and SLOSSON (3), however, reported that in alfalfa plants the sodium chloride content decreased as the sodium chloride concentration was increased in the solution. The sodium content of the sodium bicarbonate series increased gradually. The higher concentrations of

sodium bicarbonate caused an increase of the sodium content to fifteen times that of the checks.

In explaining the larger ash and sodium content of the sodium chloride over the sodium bicarbonate series, it should be remembered that the higher conductance of the combined ions of sodium and chloride over those of sodium and bicarbonate would give them greater mobility which should tend to increase their diffusion into the plant tissue. The addition of sodium bicarbonate also increased the pH values, which would promote formation of insoluble compounds of iron, calcium, magnesium, phosphate, and carbonate which would tend to lower the ash content of these plants.

The calcium content of plants in the sodium chloride series, although quite irregular, is in general higher than in the check plants. In the sodium bicarbonate series, there is a rapid decrease in the calcium content up to a concentration of 200 parts per million. The calcium content remains nearly constant at values of at least one-fourth below the checks until a concentration of 2800 parts per million has been reached, at which point it undergoes a marked decrease to approximately one-half that of the checks.

The magnesium content of the plants treated with sodium chloride showed little change throughout the whole series. In the sodium bicarbonate series there was an increase in magnesium in the lower concentrations up to 600 parts per million. There was a rather gradual decline in the magnesium content of the plants from 600 to 3000 parts per million of sodium bicarbonate.

The increase in the magnesium and the decline in the calcium content upon the addition of 100 and 200 parts per million of sodium bicarbonate could possibly be explained by the fact that calcium carbonate is more insoluble than magnesium carbonate, and that under these conditions magnesium is absorbed to replace the unavailable calcium.

The increase in the calcium and the decrease in magnesium content of the plants receiving sodium chloride solutions is in agreement with the work of CAROLUS (**4**) on the absorption of these bases in the presence of sodium. The irregular trend of the silica content in the plants watered with sodium chloride solutions prevents it from giving any useful information. Plants treated with sodium bicarbonate solutions, however, showed a pronounced increase in silica content after a concentration of 1800 parts per million had been reached. It is interesting to note that at this concentration the pH has been raised to a value of 7.3. This increased pH would tend to render the silica soluble as sodium silicate, and thereby account for the increased silica content.

Summary

The conclusions to be drawn from the sand culture experiment where sodium chloride and sodium bicarbonate in varied concentrations from 100

to 3000 parts per million were added to tomatoes may be summarized as follows:

1. The data indicate that injuries were gradually accentuated as concentrations were increased. The sodium bicarbonate produced injuries at lower concentrations, and, in general, was more toxic than the sodium chloride.

2. Under the conditions used, concentrations of either salt above the range 1400–1600 parts per million proved to be extremely toxic.

3. The ash and sodium content of the plants increased more rapidly and reached higher values in the plants treated with sodium chloride than in those treated with sodium bicarbonate.

4. Treatment with sodium bicarbonate reduced the calcium content very markedly from one-fourth to one-half that of the checks.

5. Plants receiving sodium chloride developed characteristic turgid, yellow leaves that curled and died.

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LITERATURE CITED

1. BASLAVSKAJA, S. S., and SYROESHKINA, S. M. Influence of the chloride ion on the content of chlorophyll in the leaves of the potatoes. Plant Physiol. **11**: 149–157. 1936.
2. BREAZEALE, J. F. A study of the toxicity of salines that occur in black alkali soils. Arizona Agr. Exp. Sta. Tech. Bull. 14. 1927.
3. BUFFUM, B. C., and SLOSSON, E. E. Alkali studies V. Wyoming Agr. Exp. Sta. 10th Ann. Report. 1–15. 1900.
4. CAROLUS, R. L. Effect of certain ions, used singly and in combination on the growth and the postassium, calcium and magnesium absorption of the bean plant. Plant Physiol. **13**: 349–363. 1938.
5. HAAS, A. R. C., and REED, H. S. Significance of traces of elements not ordinarily added to culture solutions for the growth of young orange trees. Bot. Gaz. **83**: 77–84. 1927.
6. HELLER, V. G., HAGEMAN, R. H., and HARTMAN, E. L.. Effect of saline and alkaline waters upon greenhouse plants. Oklahoma Acad. Sci. **20**. 1940.
7. LIPMAN, C. B., DAVIS, A. R., and WEST, E. S. The tolerance of plants for NaCl. Soil Sci. **22**: 303–322. 1926.

INFLUENCE OF AUXINS ON REPRODUCTION OF *LEMNA MAJOR*

NORMAN ASHWELL CLARK AND ELMER E. FRAHM

(WITH ONE FIGURE)

At the present time there exists a large amount of evidence showing that auxins control many of the functions of green plants. Particular attention, from both qualitative and quantitative standpoints, has been paid by investigators to the effect of the presence of auxins in the plant on the elongation of cells, the growth of roots, and the increase in the vegetative parts (**1, 8, 15**).

The addition of prepared auxin material, supplied in various forms and in various ways, also has a marked effect on plants. ZIMMERMAN and WILCOXON (**16**) found that adventitious roots were initiated and stems and leaves were affected. THIMANN and LANE (**13**) have shown that in Avena and Triticum the addition of auxin increased the dry weight, accelerated vegetative growth, and slightly hastened flowering. LOEHWING and BAUGUESS (**9**) applied heteroauxin to the soil as well as to the plants and produced variation in growth.

There are also indications that cell division and reproduction in plants are influenced by different auxins. JOST in 1935 (**7**) investigated the effect of several growth-promoting substances on cell division. Recently PRATT (**11**) checked the influence of three synthetic chemicals on the reproduction of the green alga, *Chlorella vulgaris*. He found that indole-3-acetic acid (heteroauxin) stimulated the reproduction of the Chlorella cells when present in the nutrient solution at a concentration of 10 p.p.m. and gave increased stimulation at 50 p.p.m. Indole-3-n-propionic acid was less stimulating, but was in turn greater than indole-3-n-butyric acid. Several other carbon compounds, including allantoin, were tried, but none caused an increase in the number of cells comparable with the three indole acids.

In these laboratories the writers have used *Lemna major* (*Spirodela polyrhiza*) as the test plant. Lemna is a water plant capable of producing flowers but usually propagating asexually. Its culture lends itself to a control as rigid as can be obtained with Chlorella. For several years it has been grown in inorganic nutrient solutions without organic matter, free from all microörganisms, and at a definite temperature both under electric light and in sunlight. When the logarithm of the number of fronds at any time, t, is plotted against the number of days, the result is a straight line which is reproducible under the same conditions. The slope of the line, K, is a measure of the speed of reproduction. Flasks can be subcultured indefinitely, and effects of variations in pH (**3**), light (**2**), organic or inorganic materials (**5**) are shown in the slope of the curve if the rate of reproduction is

changed, and in the form or size of the fronds if the vegetative material is affected.

The effects of three synthetic compounds on Lemna were investigated and are reported here: indole-3-acetic acid (heteroauxin), phenylacetic acid, and phenylpropionic acid.[1] Phenylacetic acid was tested at the Boyce Thompson Institute by HITCHCOCK and ZIMMERMAN (**6**) and more recently by THIMANN and SCHNEIDER (**14**) in studying the relative activities of different auxins.

The general technique for the growth of the Lemna has been given previously. The plants, free from microörganisms for hundreds of generations, were grown in an inorganic solution of purified salts in Erlenmeyer flasks at 25° C. ± 0.5° C. Solutions were sterilized at 20 lb. pressure for 15 min. and the test material was added either before sterilization or after passing through a Pasteur-Chamberland filter. The results from the two methods of sterilizing test materials indicated heat stability at the low concentrations used. The plants, after growing in the solutions, were checked each week for freedom from microörganisms and if contaminated, the culture was discarded.

The pH of the inorganic solutions was 4.7 to 4.8. This is the optimum pH for Lemna reproduction under the conditions used (**3**). SKOOG (**12**) found that plants made a measurable absorption of auxin from low concentrations in solutions at or below pH 5. The addition of small amounts of organic material did not change the pH of the Lemna cultures appreciably.

Light was supplied by electric lamps for 14½ hours daily at 400 to 500 foot-candles intensity. As the light varied somewhat in different experiments owing to the age of the electric bulbs, the rate of reproduction, K, of the plants varied slightly. Two or three cultures as controls were included in each experiment, and consisted of the Lemna in the standard inorganic medium. In the tables K is the slope of the curve multiplied by 100, and indicates the speed of reproduction.

In the first series, water solutions of the synthetic compounds were sterilized by passage through a Pasteur-Chamberland filter and were added, under sterile conditions, to the autoclaved inorganic solutions. The Lemna plants were transferred twice a week to fresh media containing the auxins.

At high concentrations (100 mg. per liter of media) all three compounds were toxic, and the Lemna died. At 10 mg. per liter both indole-3-acetic and phenylacetic also killed the plants, but phenylpropionic at that concentration produced small and light-colored fronds, with roots reduced to short stubs. At 1 mg. per liter, the rate of reproduction was slow and there was a marked shortening of the roots with all three acids; but the vegetative

[1] We are indebted to Dr. R. H. MANSKE of the National Research Council at Ottawa, Canada, for the indole-3-acetic acid; the other two are commercial preparations.

characters varied. The indole-3-acetic produced small fronds, light green, with long strands connecting mother and daughter fronds. On the other hand, the phenylacetic increased the size of the fronds, and the color compared to the standards, was darker. Phenylpropionic at this concentration had no effect on either size or color. Similar results were obtained with the concentration at 0.1 mg. per liter.

The changes caused by these last two concentrations were not permanent and the cultures recovered when transferred to the standard inorganic medium. In 4 weeks the fronds appeared normal, with roots of the usual size and reproduction approached the regular rate. Cultures from phenylacetic and phenylpropionic acids, when recovering in the standard solutions, produced fronds of large size, but these did not reproduce large daughter fronds.

Table I shows the compounds and their concentration from 0.01 to 0.0001 mg. per liter of media, the rate of reproduction K, and the effect on the fronds. The plants in the inorganic control solutions without additions are described as normal. These fronds are of good size (somewhat larger than usually found in ponds), with many well-developed roots, and have a deep green color and healthy appearance. The average of duplicates is given; K usually varied only 0.1 to 0.2.

TABLE I

RATE OF REPRODUCTION OF LEMNA WITH ADDITIONS OF AUXINS TO THE MEDIA

COMPOUND	CONCENTRATION	K	CONDITION OF PLANTS
	mg./liter of media		
(Control)	0.00	8.5	normal
Indole-3-acetic	0.01	8.6	fronds slightly lighter green, roots somewhat shorter
	0.001	8.4	normal
	0.0001	8.5	"
Phenylacetic	0.01	8.8	normal
	0.001	9.0	"
	0.0001	8.8	"
Phenylpropionic	0.01	8.9	roots slightly shorter
	0.001	9.0	normal
	0.0001	8.7	"

With these low concentrations there was little or no increase in the rate of reproduction, and as little effect on the fronds. The 0.01 mg. per liter concentrations of indole-3-acetic acid and phenylpropionic caused some inhibition of root growth, but this effect was serious only at higher concentrations. Toxic properties of the indole-3-acetic acid began to show at 0.1 mg. per liter. PRATT (**11**) found his maximum increase in reproduction of

algae at 50 mg. per liter. The difference may lie in the effect on the root system.

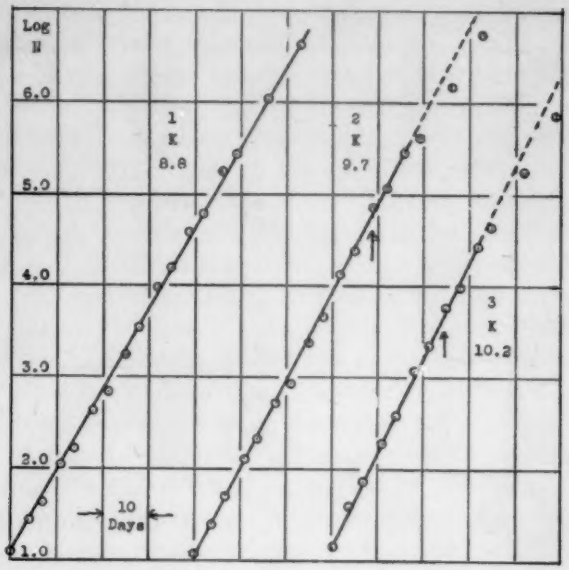

FIG. 1. Log. of number of plants on days
1. Standard control. K (slope $\times$ 100) = 8.8
2. Intermittent treatment with indole-3-acetic acid. $K = 9.7$
3. Intermittent treatment with phenylacetic acid. $K = 10.3$
↑ Treatment discontinued.

MACHT and GRUMBEIN (10) reported a definite stimulation of the roots of *Lupinus albus* seedlings after short exposure to very dilute solutions of beta-indolyl acetic acid. With the Lemna, it was observed that plants which had been treated with phenylacetic acid and phenylpropionic acid increased in size when placed in standard solutions. A further experiment was therefore planned in which the same three compounds were applied intermittently to the plants.

In this second series the Lemna plants were transferred each week to fresh standard media and the plants counted and checked for sterility as before. Each day, excluding Sundays, the plants in all cultures, except the controls, were transferred aseptically to solutions of the synthetic compounds, which were sterilized by passing through Pasteur-Chamberland filters. The Lemna remained in the solutions for 30 minutes on the laboratory table and were then returned to the standard salt solutions.

Table II shows the concentrations of the solutions, the rate of reproduction K, and the condition of the plants. The Lemna in the inorganic control solutions, without additions, are again described as normal and the test plants compared with these. Duration of the experiment was 42 days.

TABLE II

EFFECT OF INTERMITTENT APPLICATION OF AUXINS ON LEMNA

COMPOUND	CONCENTRATION	*K*	CONDITION OF PLANTS
	mg./liter media		
(Control)	0.00	8.6	normal
Indole-3-acetic	10.0	8.5	small fronds, short roots
	2.0	9.0	normal
	1.0	9.6	"
	0.5	9.5	"
	0.1	9.5	"
Phenylacetic	10.0	8.5	"
	5.0	9.5	"
	1.0	10.0	"
	0.1	10.6	fronds normal, root length increased
Phenylpropionic	10.0	10.0	normal
	1.0	10.0	"
	0.1	10.6	"

The periodic application produced marked increases in the rate of reproduction at concentrations which were toxic when the auxins were mixed with the standard medium. There is a greater difference than expected, especially in view of the fact that SKOOG (**12**) found that the activity of the indole-3-acetic decreased rapidly in a nutrient solution. He found about half the activity disappeared in 10 hours and almost all in 24 hours whether plants were present or absent.

A final experiment was carried out in order to check the results of this intermittent application and to compare the sterilization of the auxin solutions by the Pasteur-Chamberland filter and by steam sterilization. The same technique was used as in the second series, and two cultures were included with the control solutions (one of which was spoiled) in which the fronds were removed from the standard media once a day and placed in 100 ml. of sterile distilled water for 30 minutes, at the same time as the test plants were in the auxin solutions. Only one concentration, 1 mg. per liter, was used for the three auxins. The plants varied very little, if any, from the normal except those given the distilled water treatment, which were distinctly lighter in color. Table III gives the treatment and the rate of reproduction.

As noted previously, no differences in rates of reproduction were found when the auxin solutions were sterilized by autoclaving and by filtration. In both table II and table III, phenylpropionic acid is as effective as phenylacetic acid, and both stimulate reproduction of the Lemna more than the indole-3-acetic acid.

TABLE III

RATE OF REPRODUCTION OF LEMNA WITH INTERMITTENT AUXIN APPLICATION
CONCENTRATION 1 MG. PER LITER
STERILIZATION BY FILTRATION OR AUTOCLAVE

COMPOUND	TREATMENT	K
(Control)	autoclaved	8.8
"	"	9.0
Standard solution—with 30 minute water treatment	autoclaved	9.0
Indole-3-acetic acid	filtered	9.7
" " " "	autoclaved	9.6
Phenylacetic acid	filtered	10.1
" "	autoclaved	10.2
Phenylpropionic acid	filtered	10.3
" "	autoclaved	10.2

When the treatments shown in table III were discontinued, the rate of reproduction gradually dropped. Figure 1 shows three growth curves from table III; the logarithm of the number of fronds is plotted on time in days. Curve 1 is the control, $K = 8.8$; curve 2 is the result of treatment with 1 mg. of indole-3-acetic acid; and curve 3 with 1 mg. of phenylacetic acid. In the last two cases the drop in the rate of reproduction is shown when the treatment was stopped.

Summary

1. Indole-3-acetic acid, phenylacetic acid, and phenylpropionic acid were tested in varying concentrations for their effect on the rate of reproduction of *Lemna major*.

2. The Lemna was grown in inorganic solutions, under controlled light and temperature, free from microörganisms.

3. When added to the media twice weekly, the sterilized auxins failed to increase the reproduction markedly at concentrations of 0.01 mg. per liter or lower, and higher concentrations produced more or less toxicity. Ten milligrams per liter or slightly above killed the plants.

4. Intermittent application of the auxins produced increased reproduction. Both phenylacetic acid and phenylpropionic acid were more effective than indole-3-acetic acid.

5. Lemna which had been subjected to the influence of the auxins, returned to the normal rate of reproduction when replaced in the standard inorganic medium.

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LITERATURE CITED

1. Boysen Jensen, P. Growth-hormones in plants. Trans. Avery, G. S., and Burkholder, P. R. New York. 1936.
2. Clark, N. A. The rate of reproduction of *Lemna major* as a function of intensity and duration of light. Jour. Phys. Chem. **29**: 935–941. 1925.
3. ————. Plant growth-promoting substances, hydrogen ion concentration, and the reproduction of Lemna. Plant Physiol. **1**: 273–279. 1926.
4. ————. Technique for the growth of Lemna under sterile conditions with controlled temperature and light. Iowa State Coll. Jour. Sci. **7**: 13–16. 1932.
5. ————, and Roller, E. M. The stimulation of *Lemna major* by organic matter under sterile and non-sterile conditions. Soil Sci. **31**: 299–309. 1931.
6. Hitchcock, A. E., and Zimmerman, P. W. Effect of growth substances on the rooting response of cuttings. Contrib. Boyce Thompson Inst. **8**: 63–79. 1936.
7. Jost, L. Wuchstoff und Zellteilung. Ber. d. bot. Ges. **53**: 733–750. 1935.
8. Kögl, F., Haagen-Smit, A. J., und Erxleben, H. Über den Einfluss der Auxine auf das Wurzelwachstum und über die chemische Natur des Auxins der Graskoleoptilen. Zeitschr. physiol. Chem. **228**: 104–112. 1934.
9. Loehwing, W. F., and Bauguess, L. C. Plant growth effects of heteroauxin applied to soil and plants. Science n.s. **84**: 46–47. 1936.
10. Macht, D. I., and Grumbein, M. L. Influence of indole-acetic, indolebutyric, and naphthalene acetic acids on roots of *Lupinus albus* seedlings. Amer. Jour. Bot. **24**: 457–460. 1937.
11. Pratt, R. Influence of auxins on the growth of *Chlorella vulgaris*. Amer. Jour. Bot. **25**: 498–501. 1938.
12. Skoog, F. Absorption and translocation of auxin. Amer. Jour. Bot. **25**: 361–372. 1938.
13. Thimann, K. V., and Lane, R. H. After-effects of the treatment of seed with auxin. Amer. Jour. Bot. **25**: 535–543. 1938.
14. ————, and Schneider, C. L. The relative activities of different auxins. Amer. Jour. Bot. **26**: 328–333. 1939.
15. Went, F. W., and Thimann, K. V. Phytohormones. New York. 1937.
16. Zimmerman, P. W., and Wilcoxon, F. Several chemical growth substances which cause initiation of roots and other responses in plants. Contrib. Boyce Thompson Inst. **7**: 209–229. 1935.

AN ESTIMATION OF THE VOLUME OF WATER MADE AVAILABLE BY ROOT EXTENSION

PAUL J. KRAMER AND T. S. COILE

Modern views on plant-soil moisture relations stress the importance of continuous elongation of roots into new regions of the soil as an important factor in making water available to plants. It was long believed that as roots absorb the available water from the soil particles with which they are in direct contact more water is made available by capillary movement from more distant soil particles. Investigations conducted during the past 15 years indicate that this belief is not true, capillary movement of water toward the roots being so slow under average field conditions that it is of negligible importance. A brief discussion of soil-water relations will show why capillary movement is relatively unimportant.

If a limited amount of water is applied to a large volume of soil a part of the soil will be wetted uniformly while the remainder is unaffected. This situation is often observed after a shower which has wetted the upper three or four inches of soil, leaving a sharp line of demarcation between the moist soil and the dry soil beneath. The movement of water in a dry soil can occur only from larger to smaller capillaries. As PURI (**6**) has indicated, water will move from a wet soil to a dry soil only if the dry soil contains some capillaries smaller than the largest ones in the wet soil which are full of water. Water movement from wet soil to dry soil continues until contact is broken between the smaller capillaries of the dry soil which are responsible for the movement. When continuity of the liquid phase is thus broken visible movement of water ceases. The swelling of certain types of soil colloids when wetted is also of importance in inhibiting water movement by reducing the size of capillaries to a diameter so small that water molecules cannot readily pass. The amount of water held by capillarity in the soil after it has been uniformly distributed by gravitational and capillary forces is usually termed the field capacity (VEIHMEYER and HENDRICKSON, **10**). Under field conditions well drained soils are usually assumed to be at their field capacity a day or two after being wetted by rain or irrigation. SHANTZ (**7**) stated that on theoretical grounds the capillary movement of water from moister to drier regions in soils at or below their field capacity would be very slow and this was experimentally verified by KEEN (**3**) and VEIHMEYER and HENDRICKSON (**9**). According to PURI (**6**) when soil moisture is restricted to capillaries formed by particles having a diameter of 0.001 mm. or smaller relatively little water is available to plants. He considers that particles coarser than clay are mainly responsible for retaining moisture available to plants, whereas clay aids in the conservation of

this moisture by reducing its rate of movement. LIVINGSTON (**4**), KEEN (**3**), SHULL (**8**), and others have pointed out that if little or no capillary water moves toward the roots then continual extension of the roots into new regions of the soil is essential to the absorption of an adequate supply of water.

While the importance of root extension in relation to absorption is becoming well recognized, no attempt has ever been made to show how much water is actually made available by the growth of roots through the soil. The recent investigation by DITTMER (**1**) of the number, size, and length of roots developed by winter rye (*Secale cereale* L.) supplied data on root extension which makes such calculations possible.

DITTMER reported that his rye plants produced an average of 3.1 miles of new roots per day during a period of four months. These roots were mostly 120 to 250 microns in diameter and covered with root hairs which were mostly 700 to 800 microns in length. It therefore seems safe to assume that these roots and root hairs contacted the soil particles in a cylinder of soil about 2 mm. in diameter and 3.1 miles long, having a total volume of approximately 15,700 ml. If the soil mass penetrated by the roots was at the field capacity a volume of water became available to the plants each day equal to the difference between the volume present at field capacity and the volume present at the wilting percentage. No data are available for the soil used by DITTMER but one of the writers has considerable data on volume-moisture relations for several types of soil.

The volume of water available for plants in two widely different textural classes of soils is illustrated by the data for a coarse sandy loam and a heavy clay soil. The mechanical composition of these soils is given in table I and the water-holding characteristics in table II. Table II gives the moisture

TABLE I

PHYSICAL CHARACTERISTICS OF SOILS

SOIL FRACTION AND PARTICLE SIZE CLASS	TEXTURAL CLASS	
	SANDY LOAM	CLAY
	%	%
Sand, 2.00 to 0.05 mm.	78.5	13.4
Silt, 0.05 to 0.002 mm.	14.5	27.7
Clay, 0.002 mm.	7.0	58.9
Total	100.0	100.0
"Total colloidal content"*	14.2	74.6
Percentage of organic matter on oven-dry basis	1.5	1.5
Volume-weight ratio	1.75	1.24

* BOUYOUCOS, G. J. Soil Sci. **26**: 233–238. 1928, includes all the clay and a part of the silt.

TABLE II

THE WATER-HOLDING CHARACTERISTICS ON A VOLUME BASIS OF THE TWO SOILS

TEXTURAL CLASS	PERCENTAGE OF ORGANIC MATTER ON OVEN-DRY WEIGHT BASIS	PERCENTAGE OF VOLUME		ML. OF WATER AVAILABLE PER LITER OF SOIL	VOLUME WATER AVAILABLE IN 15,700 ML. SOIL
		MOISTURE EQUIVALENT	WILTING PERCENTAGE		
	%	%	%	*ml.*	*ml.*
Sandy loam	1.5	15.0	4.8	102	1,601
Clay	1.5	46.0	27.5	185	2,904

equivalents and wilting percentages calculated on a volume basis, the volume of available water per liter of soil and the volume which would be available in the soil mass penetrated each day by the roots. The volume of available water is assumed to be the difference between the volumes of water at the wilting percentage and at the field capacity. There are 102 ml. of available water per liter of sandy loam soil and 185 ml. of available water per liter of clay soil. Assuming the addition of 3.1 miles of new roots per day, 1.6 liters of water would have been made available to the rye plant in the sandy loam soil and 2.9 liters in the clay soil. No information is available concerning the actual transpiration rate of the rye plant, but it had 50 square feet of leaf surface, including both sides, or less than one square meter if only one surface was considered. MILLER (5) reported that in July the daily transpiration per square meter of leaf surface was over 1300 ml. for corn and over 2000 ml. for milo. The transpiration rate of rye growing in the greenhouse in the winter was doubtless much lower and it therefore seems highly probable that the daily root elongation alone made available sufficient water to supply the requirements of the plant. It should also be remembered that 3.1 miles of roots was merely the average addition per day. Since only a few feet of roots were added during the first week the daily increase in length was much in excess of 3.1 miles during the latter part of the experiment.

Continuing with the assumption that 3.1 miles of new roots were added each day the volume of new soil occupied daily amounted to over 30 per cent. of the total volume of soil in which the plant was growing. It therefore seems obvious that during the latter part of this experiment the new roots were crossing and recrossing older roots and thus daily reoccupying the same soil mass. This idea is also supported by the fact that at the conclusion of the experiments the total volume of roots and root hairs appears to have amounted to over one-sixth of the volume of the soil mass in which they were growing. The total surface of roots and root hairs was estimated

to be 6,875.4 square feet. How much of this surface was in actual contact with soil particles is unknown. Since the number of root hairs ranged from 19 to 53 per mm. of root it seems probable that adequate contact was made with all soil particles lying within the zone occupied by the root hairs to insure absorption of all the available water.

It is realized that this is a somewhat hypothetical example. The number and length of roots and rate of growth probably would not be the same in a sandy loam soil and in a clay soil. In spite of their imperfections, however, these calculations emphasize the fact that root extension may make available considerable quantities of water. For every 100 feet of roots added to the rye plant about 9.5 ml. of water would become available in the sandy loam soil and about 17.7 ml. in the clay soil. DITTMER cited evidence indicating that other species of grasses may have equally extensive root systems. In a more recent study (**2**) he reported that a cubic inch of soil contained 630 feet of roots and root hairs of oats. A similar volume of soil contained over 4000 feet of roots and root hairs of Kentucky bluegrass while a cubic inch of soil under rye contained 1300 feet of roots and root hairs. Such complete penetration probably does not occur with many plants other than grasses. The problem of root growth as related to the absorption of water deserves further investigation. In future studies it would be desirable to obtain data concerning the water-holding characteristics of the soil and the transpiration rate of the plants so that the rôle of root extension in supplying water could be more accurately determined.

Summary

The volume of water made available daily by root growth was calculated for winter rye using the data published by DITTMER. It was assumed that the roots contacted all soil particles in a cylinder 2 mm. in diameter and that 3.1 miles of roots were added daily. This amount of root extension would make available about 1.6 liters of water daily in a sandy loam soil at field capacity and about 2.9 liters in a heavy clay soil. It appears that at least under some conditions root extension might supply all the water required by a plant.

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LITERATURE CITED

1. DITTMER, H. J. A quantitative study of the roots and root hairs of a winter rye plant (*Secale cereale*). Amer. Jour. Bot. **24**: 417–420. 1937.

2. ————————. A comparative study of the subterranean members of three field grasses. Science, n. s. **88**: 482. 1938.
3. KEEN, B. A. The limited rôle of capillarity in supplying water to plant roots. Proc. First Int. Congress Soil Sci. **1**: 504–511. 1927.
4. LIVINGSTON, B. E. Plant water relations. Quarterly Rev. Biol. **2**: 494–515. 1927.
5. MILLER, E. C. Plant physiology. Second edition. McGraw-Hill. 1938.
6. PURI, A. N. Physical characteristics of soils: V. The capillary tube hypothesis of soil moisture. Soil Sci. **48**: 505–520. 1939.
7. SHANTZ, H. L. Drought resistance and soil moisture. Ecology **8**: 145–157. 1927.
8. SHULL, C. A. Absorption of water by plants and the forces involved. Jour. Amer. Soc. Agron. **22**: 459–471. 1930.
9. VEIHMEYER, F. J., and HENDRICKSON, A. H. Soil-moisture conditions in relation to plant growth. Plant Physiol. **2**: 71–82. 1927.
10. ———————— and ————————. The moisture equivalent as a measure of the field capacity of soils. Soil Sci. **32**: 181–193. 1931.

DETERMINATION OF THE NITROGEN FRACTIONS IN ATLAS AND MILO SORGHUM PLANTS[1]

JAMES E. WEBSTER AND HOWARD MITCHELL

(WITH ONE FIGURE)

Introduction

During the course of experiments conducted to find an explanation for the varying susceptibility of sorghum varieties to chinch bug injuries, considerable evidence has been collected indicating that nitrogen may be one of the factors involved in the problem. It is a known fact that, in general, additions of nitrogen fertilizers to plants increase the susceptibility of such plants to insect injury (**3**); DAHMS has confirmed this fact for sorghums (**1**). Our work on the chemical composition of various varieties has shown that susceptible varieties generally have a higher nitrogen content than non-susceptible varieties grown under similar conditions. Particularly is this true of field-grown Atlas and Dwarf Yellow Milo plants, which are representative, respectively, of highly resistant and extremely susceptible varieties. The study was undertaken to determine whether certain nitrogen fractions were present in greater abundance in one variety than in the other, such information being desired for cultural solution studies dealing with insect susceptibility of plants, and in the preparation of solutions for culturing chinch bugs.

Material and methods

Most workers now seem agreed that the use of fresh plant tissue is essential for study of the nitrogen fractions in plants, and this view has been adopted for our work. The Atlas and Milo plants were grown on a sandy soil in adjacent plots. The plants, which were grown at some distance from the laboratory, were cut at the surface of the ground and transported at once to the laboratory. Here they were immediately ground in a power meat grinder, and samples taken for fractiona.ion, solids, and total nitrogen. The total time which elapsed between cutting and sampling in no instance exceeded one hour, and was often somewhat less. Large samples of plants were secured, varying from many hundreds of plants when small, to at least fifteen for samplings later in the season. Plants were cut at 12:30 P.M. except for one sample cut at 10:00 A.M. The methods employed were modifications of the procedure of DAVIDSON and SHIVE which are adapted to cyanogenetic plants (**2**).

Precautions were taken to prevent contamination by ammonia in the air,

[1] Published with the approval of the Director of the Oklahoma Agricultural Experiment Station.

and blanks were also run as checks on all determinations, to further eliminate this factor.

CYANOGENETIC NITROGEN.—Fifty-gram samples of the ground tissue were placed in liter Erlenmeyer flasks containing 200 ml. of boiling water, and aerated for three hours; the HCN was collected in 5 per cent. NaOH and estimated by titration with silver nitrate. The samples were then filtered, and the residues transferred to large mortars and ground for ten minutes, then boiled and filtered. The grindings, boilings, and filtrations were repeated until the combined extracts measured about 900 ml. The extracts were then heated to boiling, 1 to 2 ml. of 10 per cent. acetic acid added, and boiling continued for one minute. The extracts were again filtered and made up to one liter. Five-hundred-ml. aliquots of these extracts were adjusted to pH of 5.5–6.0, 0.05 gm. of emulsin added, the samples heated to 50° C., and aerated and titrated as before. The two values for cyanides were combined, and the results expressed as cyanogenetic nitrogen.

SOLUBLE NITROGEN.—Aliquots of the filtered solutions were evaporated nearly to dryness in Kjeldahl flasks, and the nitrogen estimated by the Kjeldahl-Gunning-Arnold method, modified to include the nitrogen of nitrates.

AMMONIA NITROGEN.—The cyanide-free solutions were made just alkaline to phenolphthalein, and aerated into standard acid for three hours.

AMIDE NITROGEN.—The ammonia-free extracts were neutralized, and enough H_2SO_4 added to make 5 per cent. solutions. These were then refluxed for two-and-one-half hours, made alkaline, and the ammonia removed by aeration as before.

HUMIN NITROGEN.—The amide-free solutions were neutralized, and then filtered using suction. After thorough washing, the precipitates were transferred to Kjeldahl flasks, and nitrogen determined by the Kjeldahl-Gunning-Arnold method. The filtrates were diluted to one liter.

BASIC NITROGEN.—One-hundred-ml. aliquots of the filtrates from the humin nitrogen determinations were acidified by the addition of 5 ml. of H_2SO_4, and 30 ml. of phosphotungstic acid precipitating solution. The samples were placed in a refrigerator for 24 hours, removed, filtered, and total nitrogen determined on the precipitates as in the humin fraction.

AMINO NITROGEN.—Aliquots of 700 ml. of the humin nitrogen filtrate were concentrated to 200 ml., and alpha amino nitrogen estimated, using the micro Van Slyke apparatus.

NITRATE NITROGEN.—Aliquots of 50 ml. were taken of the humin filtrates, diluted with 200 ml. of water, and nitrates estimated by the Devarda method.

TOTAL NITROGEN.—The Kjeldahl-Gunning-Arnold procedure was used on 10 gm. samples of the freshly ground tissues.

Data and discussion

The data collected are presented in table I and in figure 1. The age of

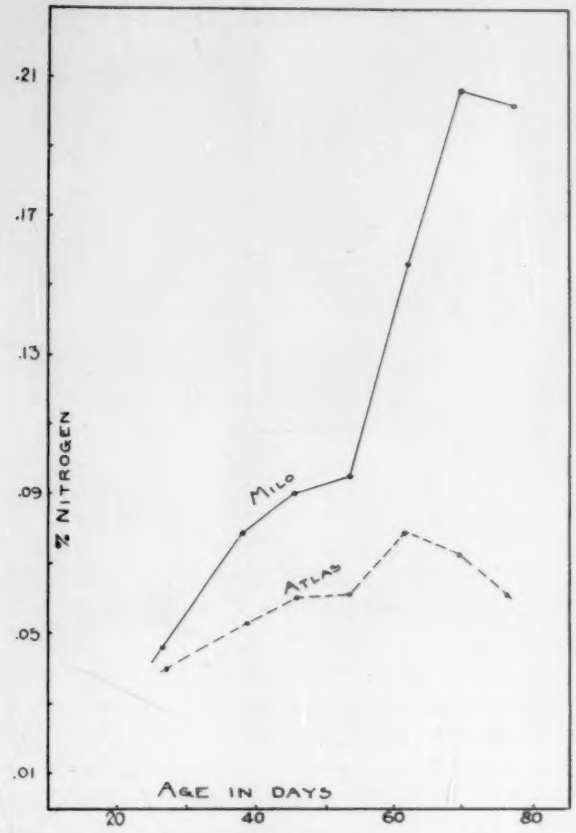

FIG. 1. Combined percentages of amino and basic nitrogen.

plants is expressed as days from planting, since the location of the field precluded daily observations to note when the shoots emerged. Attention is called to the fact that during the early stages of growth, Milo plants were larger and more vigorous than Atlas plants, and that it was not until after the third sampling (46 days) that Atlas plants exceeded the Milo plants in size, although Milo is called a dwarf variety and Atlas a tall variety.

SOLUBLE NITROGEN.—A major difference is noted here in that generally the soluble fraction in Milo increases as the plants age, while in Atlas this fraction fluctuates but little and at the last sampling reaches its lowest point. This is of interest, for it is the soluble fraction that would be expected to furnish the nitrogen available to the chinch bugs.

Cyanide, ammonia, humin, and nitrate nitrogen percentages, while varying some at specific dates and in total amounts, are all relatively low, and show the same general trends for both varieties.

TABLE I

NITROGEN FRACTIONATION IN SORGHUM PLANTS
FIELD GROWN (1939)

AGE	HEIGHT	SOLIDS	NITROGEN									
			TOTAL	INSOLUBLE	SOLUBLE	CYANIDE	NH_3	AMIDE	HUMIN	BASIC	AMINO	NITRATE
days	*in.*	%	%	%	%	%	%	%	%	%	%	%
MILO												
28	7	18.60	0.470	0.364	0.106	0.0054	0.0003	0.0000	0.0060	0.0140	0.0362	0.016
38	12	20.35	0.432	0.320	0.112	0.0041	0.0005	0.0003	0.0072	0.0404	0.0390	0.000
46	15	17.52	0.432	0.302	0.130	0.0054	0.0005	0.0000	0.0036	0.0445	0.0451	0.024
53*	20	17.48	0.412	0.291	0.121	0.0023	0.0001	0.0003	0.0116	0.0584	0.0360	0.032
61‡	29	18.02	0.419	0.248	0.171	0.0027	0.0003	0.0000	0.0056	0.0804	0.0753	0.028
69	36	21.64	0.442	0.252	0.190	0.0026	0.0013	0.0000	0.0032	0.0904	0.1153	0.008
76†	42	27.21	0.508	0.265	0.243	0.0026	0.0017	0.0000	0.0000	0.0624	0.1408	0.008
ATLAS												
28	5	21.26	0.455	0.352	0.103	0.0072	0.0007	0.0030	0.0088	0.0090	0.0331	0.008
38	10	20.83	0.406	0.308	0.098	0.0042	0.0000	0.0055	0.0080	0.0304	0.0240	0.000
46	15	14.70	0.370	0.258	0.112	0.0032	0.0011	0.0000	0.0092	0.0340	0.0271	0.008
53*	28	13.10	0.322	0.210	0.112	0.0025	0.0005	0.0031	0.0056	0.0384	0.0240	0.020
61‡	42	12.50	0.279	0.176	0.103	0.0018	0.0013	0.0051	0.0024	0.0504	0.0295	0.012
69	50	15.32	0.290	0.195	0.095	0.0016	0.0011	0.0005	0.0056	0.0324	0.0404	0.008
76†	60	21.39	0.279	0.201	0.078	0.0007	0.0009	0.0014	0.0000	0.0280	0.0329	0.004

* Atlas plants now exceed Milo in diameter also.
† Milo fully headed; Atlas just beginning.
‡ Plants cut at 10:00 A.M.

Amide nitrogen is found more abundantly in Atlas plants; on the basis of the small amounts present, however, it is not considered significant.

Basic nitrogen percentages are much larger than those of the previous soluble fractions considered, and here we find that the Milo percentages are consistently higher, although showing the same seasonal trend that is found in Atlas plants.

Amino nitrogen percentages show by far the greatest varietal differences, and it is in this fraction, combined with the basic fraction (largely diamino acids) (**4**), that we see an explanation for the higher percentages of soluble nitrogen found in the Milo plants. This difference is more strikingly shown by referring to the graph, figure 1, where these two values for amino nitrogen have been combined. This shows not only the much greater percentages found in Milo plants, but also some differences in seasonal trends. It is thus apparent that, disregarding some small individual variations, most of the differences in soluble nitrogen content can be accounted for by variations in the amino nitrogen content.

Summary

Field grown Dwarf Yellow Milo plants are shown to be higher in nitrogen content than Atlas plants.

Soluble nitrogen generally increases in the growing Milo plants and remains constant or decreases in the Atlas plants.

Amide nitrogen is practically absent in growing milo plants.

Most of the observed differences in soluble nitrogen content between the Dwarf Yellow Milo plants (chinch bug susceptible), and Atlas (resistant) can be accounted for in the basic and alpha amino nitrogen fractions.

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LITERATURE CITED

1. Dahms, Reynold G. Personal communication to the authors. Assistant in Entomology, located at Lawton, Oklahoma.
2. Davidson, O. W., and Shive, J. W. Determination of the nitrogenous fractions in vegetative tissue of the peach. Plant Physiol. **10**: 93–114. 1935.
3. Martin, Hubert. The scientific principles of plant protection. 2nd ed., Edward Arnold and Co., London, 1936.
4. Murneek, A. E. Biochemical studies of photoperiodism in plants. Missouri Agr. Exp. Sta. Res. Bull. 268. 1937.

SOME EFFECTS OF STANNOUS SULPHATE AND STANNIC CHLORIDE ON SEVERAL HERBACEOUS PLANTS

BARNEY BARNETT COHEN

(WITH THREE FIGURES)

Introduction

Until recently it was thought that boron, manganese, copper, zinc (**4**), and iron (**7**) were the only essential trace elements necessary for plant growth. Investigators now believe that other elements also are essential. YOUNG (**14**) observed that additions of rare elements, including tin, stimulated the growth of timothy and algae. ARNON (**2**) suggested that such elements as Mo, V, Ti, W, Cr, Cd, and Co are essential to maximum growth of lettuce and asparagus. MICHEELS (**9**) reported that solutions of colloidal tin had a marked effect upon the early growth of wheat, oats, peas, and buckwheat. ALLISON, HUNTER, and BRYAN (**1**) noted slight favorable responses with additions of tin and other elements in sixty crop plants grown on the peat soils of the Florida everglades. In growing wheat, rye, maize, and tobacco in water culture SCHROPP and SCHARRER (**11**) found that traces of tin chloride with other elements induced favorable responses.

In the experiments reported here, stannous sulphate was added to nutrient solutions and observations were made of its effects upon corn and peas. The effects of varying concentrations of stannic chloride were observed for the sunflower only.

Procedures

Sunflowers, corn, and garden peas were grown in glazed pots, six plants to each pot. Quartz sand was used as the growth medium. The cultures were supplied with nutrient solutions twice daily, and flushed twice weekly with distilled water to prevent accumulation of salts in the sand. Solutions were prepared from half-molar stock solutions of calcium nitrate, dipotassium phosphate, and magnesium sulphate, purified as first suggested by STEINBERG (**12**) with the modifications adopted by ARNON and STOUT (**3**). Calcium carbonate (65 gm.) was added to 5 liters of stock solution, which was then autoclaved for 20 min. at 15 lb. (120.5° C.). Nutrient solutions were made from the purified stocks by diluting 12 ml. of calcium nitrate, 9 ml. of dipotassium phosphate, and 9 ml. magnesium sulphate with distilled water (copper still) to 1 liter. In addition to the trace elements, Cu, B, Mn, and Fe, tin in the form of stannous sulphate was supplied to the nutrient in concentrations of 0.2, 1, 5, 20, and 100 p.p.m. In the sunflower tests, tin was added as stannic chloride in concentrations of 0.01, 0.05, 0.1, 0.5, 1, 10, and 100 p.p.m. No attempt was made to purify the trace element solu-

tions since these were present only in minute quantities. Stock solutions were brought to pH 6.5; and to prevent contamination, glassware was cleaned with 25 per cent. HCl.

A method of detecting tin in distilled water was necessary because the distilled water used was carried in tin piping. The flame color test suggested by MEISSNER (6) and FEIGL (5) was used. To increase the sensitivity of the test, the test-water was reduced to one-half its volume by boiling. This concentrated sample was then acidified strongly with HCl, and a few pieces of zinc added. A test-tube containing cold water was used to stir this mixture; it was then placed in a nonluminous bunsen burner flame. In this test the blue mantle which forms around the test-tube in the presence of tin was absent, thus eliminating tin as a contaminant of the distilled water to one part in three million. Distilled water leached through the sand and recovered also failed to show the tin test.

To detect the presence of bivalent tin in plant tissues, the spot test method suggested by FEIGL (5) was modified. Thin sections of plant stems were acidified in 20 per cent. HCl by dropping the acid upon a glass slide holding the sections. A drop of dilute ferric chloride, and after a few minutes, a crystal of tartaric acid were added. When the tartaric acid crystal had dissolved, a drop of dimethylglyoxime was used on the sections, followed by ammonia. A red coloration, developed in the presence of reduced iron, indicates that stannous tin is present in the plant tissues.

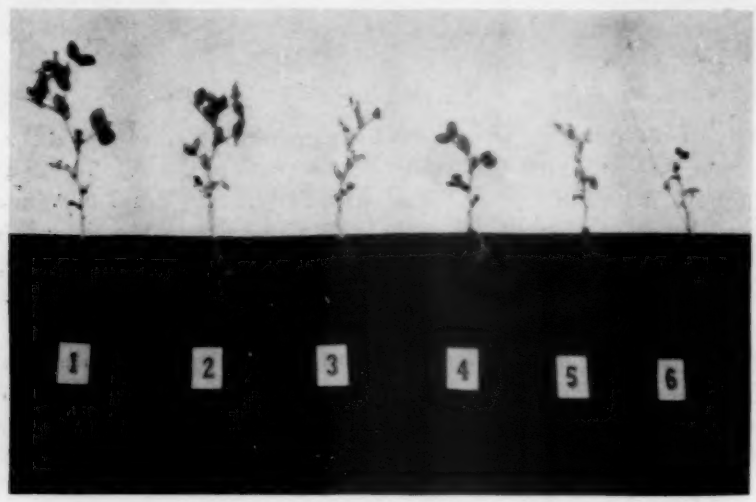

FIG. 1. The effect of increasing concentrations of stannous sulphate on peas.

1. Control
2. 0.2 parts per million
3. 1.0 p.p.m.
4. 5 p.p.m.
5. 20 p.p.m.
6. 100 p.p.m.

Results

Figure 1 shows the effect on peas of stannous sulphate in concentrations from 0.2 to 100 p.p.m. The control, which was without tin sulphate, was slightly taller than plants grown in 0.2 p.p.m., although the difference is not marked since the branches and leaves were similar. One interesting result was that plants supplied with 0.2 p.p.m. of stannous sulphate showed increased root growth. Concentrations from 1 to 100 p.p.m. caused toxicity symptoms. These were reduced growth of internodes, reduction in size of roots, reduction in number of roots, absence of flowers in concentrations above 20 p.p.m., and yellowing of the lower leaves.

Figure 2 shows the effect of stannous sulphate on corn. The control, 0.2 p.p.m., and 1 p.p.m. plants were similar in structure, although the one receiving 1 p.p.m. appeared to be more vigorous. Other effects noted were a deeper chlorophyll green, and increased height. The plants which had received 0.2 and 1 p.p.m. of stannous sulphate (2 and 3, fig. 2) showed an increase in root growth. Greater concentrations (5, 20, and 100 p.p.m.) caused stunting and chlorosis, especially a yellowing of the lower leaves.

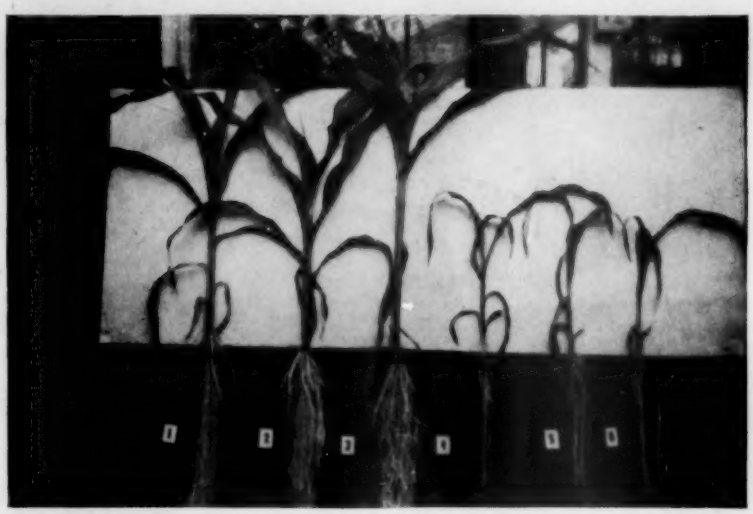

FIG. 2. The effect of stanous sulphate on corn.

1. Control
2. 0.2 parts per million
3. 1.0 p.p.m.
4. 5 p.p.m.
5. 20 p.p.m.
6. 100 p.p.m.

Figure 3 illustrates the effect of stannic chloride on the sunflower. The results with low concentrations (0.01, 0.05, and 0.1 p.p.m.) show that stannic chloride has a pronounced effect upon the growth of the sunflower. It is particularly effective on the roots, which were about twice the size of those

of the control. With additions of tin chloride above 1.0 p.p.m., stunting and reduced growth of leaves, internodes, and roots were observed. In concentrations of 100 p.p.m. sunflower was unable to reach a height of more than 12 in.

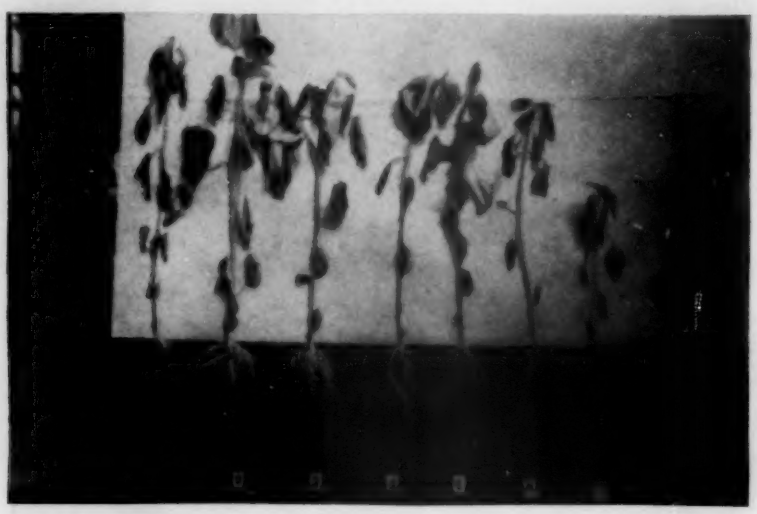

FIG. 3. The effect of stannic chloride in concentrations from 0.01 to 100 p.p.m. on the sunflower.

1. Control
2. 0.01 parts per million
3. 0.05 p.p.m.
4. 0.1 p.p.m.
5. 0.5 p.p.m.
6. 1 p.p.m.
7. 10 p.p.m.
8. 100 p.p.m.

Discussion

From the results obtained with trace concentrations of tin sulphate, it appears that its effects are limited to slight increases in the root growth of corn and pea. It also appears that corn and pea react specifically to this compound. In the corn plant 1 p.p.m. of stannous sulphate caused no unfavorable nor abnormal growth responses, while in the pea plant there was a decrease in the size, number of roots, and a stunting or top growth with this concentration. In concentrations of 100 p.p.m. the pea plant was barely able to exist; while corn, although stunted, was about the same size as the corn plants grown in much lower concentrations. As there are two nutrient ions to be considered, the slight stimulative effect on root growth might be caused by an increase in the concentration of the sulphate ion, but the added concentration in some cases is very small in proportion to the sulphate ion already present in the solution. The responses to high concentrations of stannous sulphate indicate the possibility that both sulphate and tin ions may cause changes in growth. It should be noted that in the higher con-

centrations there was a tendency toward precipitation of the tin salt. In the sunflower, trace additions of tin chloride caused striking changes in root growth, to about twice the size of the controls. The lack of marked favorable responses in root growth with additions of stannous sulphate to pea and corn may indicate that in some way the sulphate ion modifies the effectiveness of the tin ion, or that the plants are less sensitive to tin than sunflowers, or it may be that the chlorine ion in the presence of tin stimulates root development of the sunflower. Since it has been discovered very recently that tin increases the growth rate of certain aquatic animals, it seems more probable that the tin ion in very low concentrations is stimulatory to root growth. TOTTINGHAM (**13**) has reported some investigations with sodium and chlorine in which he found increased root development in the sunflower plant. He suggests that chlorine may function in some plants as a nutrient. This possibility must be kept in mind in interpreting the response of sunflowers to stannic chloride.

Conclusions

1. A method for the detection of bivalent tin in plant tissues has been devised.

2. In the sunflower, 0.01 and 0.05 p.p.m. of tin as tin chloride stimulates root growth.

3. Concentrations of tin, either as chloride or sulphate, in concentrations of 5 or more p.p.m. have toxic effects upon corn, pea, and sunflower.

The writer expresses his appreciation to Dr. C. A. SHULL for suggestions made in connection with this study.

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LITERATURE CITED

1. ALLISON, R. V., BRYAN, O. C., and HUNTER, J. V. The stimulation of plant response on the raw peat soils of the Florida everglades through the use of copper sulphate and other chemicals. Florida Agr. Exp. Sta. Bull. 190. pp. 33–80. 1927.
2. ARNON, D. I. Micro-elements in culture-solution experiments with higher plants. Amer. Jour. Bot. **25**: 322–325. 1938.
3. ————————, and STOUT, P. R. Experimental methods for the study of the role of copper, manganese, and zinc in the nutrition of higher plants. Amer. Jour. Bot. **26**: 144–149. 1939.
4. BRENCHLEY, W. E. The essential nature of certain minor elements for plant nutrition. Bot. Rev. **2**: 173–196. 1936.
5. FEIGL, F. Spot tests and color reactions as methods of microchemical work. Translated by JANET W. MATTHEWS. Nordemann Pub. Co., New York.

6. Haas, A. R. C., and Reed, H. S. Significance of traces of elements not ordinarily added to culture solutions for growth of young orange trees. Bot. Gaz. **33**: 77–84. 1927.
7. Jones, H. W., and Shive, J. W. The influence of iron in the forms of ferric phosphate and ferrous sulphate upon the growth of wheat in nutrient solution. Soil Sci. **11**: 93–101. 1921.
8. Meissner, H. Ein neuer Nachweis für Zinn. Zeitschr. Ann. Chem. **80**: 247–252. 1930.
9. Micheels, H. Sur les stimulants de la nutrition chez les plants. Rev. Sci. **14**: 427–429. 1906.
10. ————————, and De Heen, P. Action de la solution colloidale d'étain sur des graines in germination. Bull. Acad. rayate Belg. Class des sci., no. 7. 1905.
11. Schropp, W., and Scharrer, K. Wasserkultur Versuche mit der "A–Z" Lösung nach Hoagland. Jahrb. wiss. Bot. **78**: 544–563. 1933.
12. Steinberg, R. A. Nutrient solution purification for removal of heavy metals in deficiency investigations with *Aspergillus niger*. Jour. Agr. Res. **51**: 413–424. 1935.
13. Tottingham, W. E. The rôle of chlorine in plant nutrition. Science n. s. **42**: 68. 1915.
14. Young, R. S. Certain rarer elements in soils and fertilizers and their rôle in plant growth. New York (Cornell) Agr. Exp. Sta. Mem. 174. 1935.

THE LITHIUM METHOD OF MEASURING THE EXTENT OF CORN ROOT SYSTEMS

J. D. Sayre and V. H. Morris[1]

Introduction

Investigations of the root systems of corn plants which involve manually tracing the extent of individual roots are tedious and expensive. During the past 5 years a method of measuring the extent and distribution of corn roots through their absorption of lithium chloride has been under investigation. The method involves the placing of small quantities of the lithium salt in the soil at marked locations between the rows of corn and then spectrographically testing the tissue of nearby plants for the presence of lithium.

Lithium is not a normal constituent of soils except in rare instances. It is not toxic to the plants and is readily absorbed and accumulated in the plant in quantities which can be detected by a spectroscope. Very dilute concentrations may be identified spectroscopically, and no other element gives a line at 6708 Å. Its similarity in chemical properties to sodium and potassium probably accounts for its ease of absorption and accumulation in the plant, since these two elements are almost universally present in plant tissues.

The extreme simplicity of the method makes it useful in certain kinds of root investigations. It is recognized, however, that the method may be somewhat restricted in application.

Experimental procedure

The procedure of laying out the plots and conducting the experiments has varied from year to year. For most of the experiments the corn plants were spaced 14 inches apart in rows 42 inches apart, but in some tests the plants were planted in hills, in circles, or in spirals. In the spirals, the plants were spaced 14 inches apart on a line formed by unwinding a cord from a stationary drum 22 inches in diameter. These different arrangements were used to obtain uniform spacing of the plants at definite distances from the lithium spot. The lithium was placed midway between the rows or at the center of the spirals.

The lithium applications in most of the tests were made about the middle of July after the cultivations that might disturb them had been completed, and at the rate of approximately 2 grams of lithium chloride to 1,000 grams

[1] Department of Agronomy, Ohio Agricultural Experiment Station, and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, cooperating.

of soil. This was about the exchange capacity of the soil. A core of soil first was removed from each point of lithium application to a depth of 18 inches with a 4-inch post auger. The lithium chloride was dissolved in a small amount of water, sprinkled on the removed soil and thoroughly mixed with it. The treated soil then was returned to the hole and carefully packed to about the same consistency as before.

Each lithium spot was marked carefully. About September first all nearby plants were collected, their distance from the lithium spot was measured, and they were tested for the presence of the element. The test consisted of burning a small piece of tissue removed from one of the lower nodal plates in a Bunsen flame in front of a spectroscope. The presence of lithium was detected by a spectral line at 6708 Å. If the plant tissue contained lithium, the roots of that plant must have penetrated the core of lithium-treated soil and absorbed some of the salt. A negative test indiated that the roots had not extended to the lithium spot, or, if so, had failed to absorb the salt and transport it to the culm. As lithium is so easily absorbed by the plant and its detection in such small quantities is possible in the spectroscope, plants giving negative tests were considered as having no roots entering the lithium core. The results were recorded only as positive or negative, indicating the presence or absence of the element. No attempts were made to estimate the density of the roots in the lithium spot by measuring the quantity of lithium absorbed by the plant.

At the end of the season a 6-inch core of soil was removed from each lithium spot to ensure as complete removal of the material as possible. A series of spectroscopic tests showed that the lithium had not moved from the spot where it was placed. It enters into the base exchange complex of the soil and does not move with the soil water.

Experimental results

In 1932, preliminary tests showed that the method would work and lateral root extensions of more than 30 inches were found. The 1933 tests indicated about 2 grams of lithium chloride to 1,000 grams of soil as a proper amount to use. The 1934 experiments indicated a need for modifying the spacing of the plants in the row. With the plants spaced 14 inches apart in the row, few occurred at distances between 28 to 35 inches from the lithium source. As this distance is about the maximum root extension of many of the plants under the conditions obtaining at Wooster, more plants were needed in this interval for a good estimate of maximum extension. In 1935 and 1936 the plants were arranged in spirals around the lithium spots in order to locate them at more uniform intervals of space from the spot. Many other tests were conducted during these years, but not all of them can be discussed here.

TABLE I

SUMMARY OF ALL DATA INVOLVING SINGLE CROSSES AND A LITHIUM CONCENTRATION OF 2 GRAMS OF THE CHLORIDE TO 1,000 GRAMS OF SOIL

Year	Lithium test	Numbers of plants yielding positive or negative lithium tests at the distances from plant to lithium indicated								
		30–39 cm. or 11.8–15.4 in.	40–49 cm. or 15.7–19.3 in.	50–59 cm. or 19.7–23.2 in.	60–69 cm. or 23.6–27.2 in.	70–79 cm. or 27.6–31.1 in.	80–89 cm. or 31.5–35.0 in.	90–99 cm. or 35.4–39.0 in.	100–109 cm. or 39.4–42.9 in.	110–119 cm. or 43.3–46.9 in.
1933	Positive	.....	6	12	2	5	0	0	0	0
	Negative	.....	1	8	11	13	2	7	19	3
1934	Positive	.....	10	15	9	2	0	0	0	.....
	Negative	.....	0	0	3	8	0	10	9	.....
1935	Positive	12	31	35	21	12	0	1	.....	.....
	Negative	0	2	20	24	32	38	43	.....	.....
1936	Positive	37	15	17	14	21	18	5	2	0
	Negative	0	0	0	3	8	9	28	10	3
Total	Positive	50	62	80	46	40	18	6	2	0
	Negative	0	3	28	41	61	47	88	38	6
Percentage	Positive	100	95	74	48	40	28	6	5	0
	Negative	0	5	26	52	60	72	94	95	100

All of the data obtained in experiments involving single crosses and a lithium concentration of 2 grams of lithium chloride to 1,000 grams of soil have been summarized in table I. This table shows the distance from the plants to the lithium and the number of plants at each distance yielding positive or negative tests. The totals for the 4 years also are expressed on a percentage basis. All plants 40 cm., or 15.7 inches, from the lithium spot contained lithium, indicating their roots had penetrated the lithium core. No plants located as much as 110 cm., or 43.3 inches, from the lithium core contained lithium, and on the basis of this test the lateral extension of their roots is assumed to be less than this distance. Half the plants had roots 60 to 69 cm., or about 27 inches, in length.

The distances indicated by the method are not as great as would be expected from some results reported by others who actually dug the plants. Perhaps these results represent the extent of the mineral absorptive capacity of the corn plant rather than the maximum extension of roots. Fertilizer placement studies with corn indicate that the greatest mineral absorption occurs very near the plant. Cultural practices in much of the Corn Belt have shown that three plants to the hill with the hills space about 3½ feet each way, or equivalent stands drilled in rows 3½ feet apart, produce the largest yields of corn. These results seem to indicate that corn roots extend somewhat more than half the distance between rows. It is possible that root competition limits their extent laterally.

No tests were conducted on the maximum depth to which the root systems extended because of the shallow nature of the soils at Wooster, which have a tight clay subsoil at about 18 inches and a layer of shale at 2½ to 3 feet.

OHIO AGRICULTURAL EXPERIMENT STATION
WOOSTER, OHIO

BRIEF PAPERS

MONTHLY ABSORPTION OF NUTRIENTS FROM THE SOIL BY THE PERFECTION PIMIENTO PLANT[1]

H. L. Cochran and L. C. Olson

(WITH ONE FIGURE)

Studies on the absorption of nutrients from the soil by the Perfection pimiento plant were begun at the Georgia Agricultural Experiment Station in the Spring of 1939. Information of this nature is desirable because a knowledge of the kind and amount of nutrient absorbed at intervals throughout the growing season gives a better understanding of the fertilizer required for a more successful cultural program.

The soil on which the pimientos used in this work were grown is Cecil sandy clay loam. This is one of the most important soil types in the Piedmont section of Georgia, and pimiento culture is largely confined to this or a closely related soil type. The mean monthly rainfall for the 1939 growing season at Experiment, Georgia, where the experimental plats were located was 4.02 inches, and the mean temperature for the same period was 73.4° F.

The pimientos used in this study were fertilized at the rate of 600 pounds per acre with a mixed fertilizer analyzing 8 per cent. nitrogen, 8 per cent. phosphoric acid, and 6 per cent. potash. This fertilizer was applied in the row and mixed well with the soil several days before the plants were set out. At 30-day intervals, after transplanting, plants were cut off just above the soil line, dried at 75° C., and ground for chemical analysis. After the first month, the buds and fruits were separated from the plants for the analysis. In collecting the samples, care was taken to select uniform plants.

The results of the absorption of nutrients, calculated on an acre basis of 4,149 plants, are shown graphically in figure 1. At the end of the first month in the field, the entire acre of pimientos absorbed from the soil less than one-half pound of nitrogen and phosphoric acid, and even smaller amounts of potash, calcium, and magnesium. The absorption of nutrients during the second month was small, but there was an increase over the absorption for the first month. The largest quantity of all nutrients was absorbed during the third month. These findings are well in agreement with those of Hester (**2**) for the tomato plant and those of Carolus (**1**) for the Irish potato. After the third month, there was a gradual decline in absorption. Larger quantities of nitrogen were absorbed than of any other nutrient. Calcium, potassium, magnesium, and phosphorus followed in this respective order as to quantity absorbed. Although phosphorus was absorbed the least of any,

[1] Published with the approval of the Director of the Georgia Agricultural Experiment Station as Journal Series Paper no. 73.

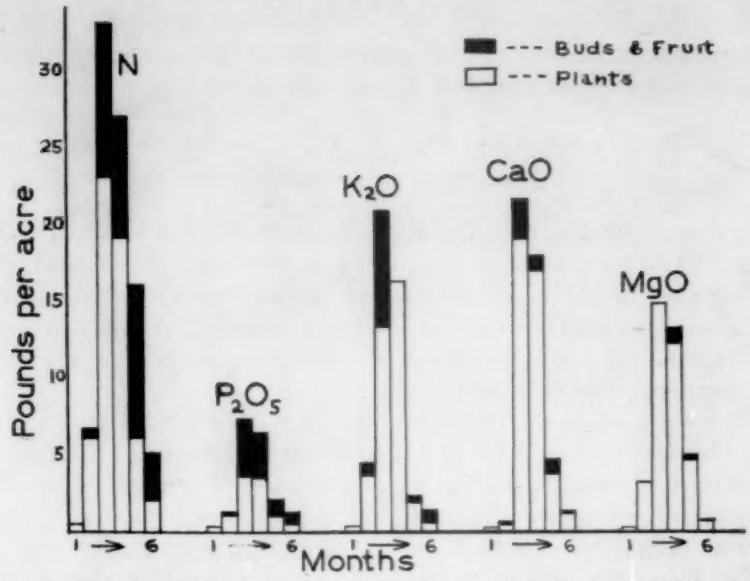

FIG. 1. The absorption nutrients by the Perfection pimiento plant at 30-day intervals during the growing season.

it is possible that rather large quantities should be included in the fertilizer mixture because of the rather high phosphate-fixing capacity of some of the pimiento soils, which makes some of the phosphate unavailable to the growing plant.

From the work completed thus far, it appears that ample plant nutrients should be made available to the pimiento plant, especially during the latter part of the second and throughout the third month after being set in the field. This may best be accomplished by placing most of the fertilizer under the plants a few days before setting and the balance as a sidedressing during late June. The second application should, perhaps, be supplemented with some extra nitrogen and potash.

DEPARTMENTS OF HORTICULTURE AND AGRONOMY
GEORGIA AGRICULTURAL EXPERIMENT STATION
EXPERIMENT, GEORGIA

LITERATURE CITED

1. CAROLUS, R. L. Chemical estimations of the weekly nutrient level of a potato crop. Amer. Potato Jour. **14**: 141–153. 1937.
2. HESTER, JACKSON B. The absorption of nutrients by the tomato plant at different stages of growth. Proc. Amer. Soc. Hort. Sci. **36**: 720–722. 1938.

NOTES

Seventeenth Annual Meeting.—Plans for the seventeenth annual meeting of the American Society of Plant Physiologists at Philadelphia in December, 1940, are well advanced. The meetings begin so late in the month that the first three days of the week, Monday to Wednesday, December 30, 1940, to January 1, 1941, have been chosen for the sessions. On Monday morning there is a symposium on Protoplasm; during the afternoon, there will be a joint session with Section G, A.A.A.S., and a parallel session for the reading of papers. This arrangement is made necessary by the large number of papers to be accommodated with time. On Tuesday morning, December 31, there is a joint session with the Phytopathological Society of America, and a parallel session for the presentation of contributions. In the afternoon, a symposium on Plant Nutrition is scheduled; this will be a joint meeting with the American Society for Horticultural Science. On Wednesday, January 1, 1941, there will be sessions, morning and afternoon, for reading of contributions. These will be divided into two groups for simultaneous meetings if necessary.

The headquarters will be at the Hotel Benjamin Franklin. The annual dinner, at which the award of the CHARLES REID BARNES life membership and the STEPHEN HALES award will be announced, has been set for Monday evening. Members and friends are urged to arrive early, and secure dinner tickets at once, in order that places may be reserved for the large number of attendants who will want to be present at these notable ceremonies. Dr. JOHN W. SHIVE will deliver the sixth STEPHEN HALES address at this time. The details as to the place of the annual dinner are uncertain, but it is hoped that the Athletic Club may be able to accommodate us with sufficient room. The program committee is to be congratulated on having its plans so well matured at this early date. There is every reason to expect a very large gathering, and members are advised to make early reservations; otherwise it may be difficult to secure hotel accommodations conveniently close to the meeting places.

Western Section.—The Western Section of the A.S.P.P. held its annual meeting at Seattle, with the summer meeting of the A.A.A.S., June 18–22, 1940. One of the outstanding features of this meeting was the symposium on Photosynthesis, which was conducted by Dr. H. A. SPOEHR, of the Carnegie Institution of Washington, D. C. Summarized briefly, this symposium included the following reports: HANS GAFFRON pointed out that, under certain conditions, oxygen is not liberated, but is used for the oxidation of organic hydrogen donors during photosynthesis. ROBERT EMERSON showed that the quantum number of the photosynthetic process is not 4, but approxi-

mately 10. WINSTON M. MANNING reported evidence that in diatoms carotenoids, in addition to chlorophylls, may be active in absorbing light energy for the photosynthetic processes. S. RUBEN reported experiments which indicated that plants reduce CO_2 in the dark by combining it with a large molecule of an as yet unidentified structure. E. D. MCALISTER showed the intimate relations between fluorescence and photosynthesis. WILLIAM ARNOLD showed that many chlorophyll molecules must be present in order to *insure* the photosynthetic process, although one individual molecule might do the work. Finally, C. B. VAN NIEL summarized the modern work on photosynthesis by presenting a scheme of the process. In this scheme CO_2 is reduced in the dark, and the H_2O (H_2A) is oxidized in the light.

In addition to this extremely valuable symposium, there was one on Aquatic Botany, at which Dr. GEORGE B. RIGG presided. GILBERT M. SMITH discussed the temperature of water in relation to the presence or absence of certain algae. JOHN RAPER presented evidence of the presence of sexual hormones in both algae and water molds. G. H. HOLLENBERG reported on the relative abundance of marine algae in relation to the presence of diatoms. LYMAN D. PHIFER discussed the distribution of marine diatoms; and J. VAN OVERBEEK showed that auxins are present in all marine algae investigated, and that traumatic acid and thiamin are growth factors for unicellular green algae. DAN BONNELL discussed the inter-relations of plant and animal life in certain fresh water lakes.

A third symposium dealt with the problems of Phosphate Nutrition. Dr. H. D. CHAPMAN was the leader of this symposium. PERRY R. STOUT illustrated by means of models how the phosphate is fixed on kaolinite and bentonite. ORLIN BIDDULPH showed how radio-active phosphorus "circulates" in the plant. The soil-plant relationships were discussed by W. T. MCGEORGE.

There were two half-day programs for short papers. Noteworthy among these were the following: J. B. BIALE reported that fungi produce emanations which affect the respiration of lemons; W. O. WILLIAMS discussed sugar in tracheids; L. A. HOHL showed that formic acid is not formed by yeast fermentation; ROBERT H. WILLIAMS gave an account of the reserve carbohydrates of algae; and ROBERT H. TSCHUDY reported studies on the control of growths on ship bottoms.

A trip to the oceanographic laboratories at Friday Harbor at the close of the meeting was very instructive, and from the number in attendance, it must be rated a great success. The total attendance at the meetings as a whole was estimated at about 100. At the annual dinner of the Western Section on Thursday evening, June 21, officers were announced for 1941 as follows: Chairman, A. S. CRAFTS, the University of California; vice-chairman, FRANK M. EATON, Rubidoux Laboratory, Riverside, California; secretary, J. VAN OVERBEEK.

Eighth American Scientific Congress.—The Eighth American Scientific Congress was held at Washington, D. C., in May, 1940. Its purposes were mainly to afford opportunities of friendly social contact between North and South American scientists, and to extend and consolidate the sense of unity and friendship existing between all of the American republics. Science, particularly, is friendly beyond the boundaries of nations. A number of papers on physiology and plant physiology were presented at the Congress, including the following: *Permeability in the nuclear membrane evidenced by intranuclear colloidal changes,* by WILLIAM R. DURYEE; *The nature of living cells as revealed by micro-operations,* by ROBERT CHAMBERS; *What the study of green plants has taught us concerning a probable mechanism of photosynthesis,* by O. L. INMAN; *Plant tissue cultures. A new technique in experimental science,* by PHILIP R. WHITE; and *Vitamins as growth substances for plants,* by WILLIAM J. ROBBINS. The contributions by our South American friends fell mainly in the fields of general botany, agriculture, and conservation.

The Congress was ably handled, and served its purposes admirably. The American Society of Plant Physiologists was represented at the Congress by Dr. CHARLES O. APPLEMAN, Professor of Plant Physiology and Dean of the Graduate School, at the University of Maryland.

Life Membership Committee.—The seventeenth award of the CHARLES REID BARNES life membership will be made at Philadelphia, the announcement being a part of the festivities of the annual dinner. President FRANK P. CULLINAN has named the committee which is to have the privilege of selecting the recipient of the award. The membership of the committee is as follows: Chairman, Dr. H. R. KRAYBILL, Purdue University; Dr. C. J. LYON, Dartmouth College; Dr. PAUL J. KRAMER, Duke University; Dr. F. H. STEINMETZ, The University of Maine; and Dr. W. E. TOTTINGHAM, The University of Wisconsin.

Finances.—At the beginning of the year 1940 it seemed that the American Society of Plant Physiologists might be facing a very trying year because of the widespread economic disturbance throughout the world. Thanks to the efforts of our secretary, Dr. W. E. LOOMIS, and great numbers of American plant physiologists who understood the situation and acted upon their knowledge, we have come through the year in sound, solvent condition. Every society that undertakes to publish a scientific journal is at the mercy of its income. Few of our societies devoted to science have adequate financial reserves and endowments to assist them in times of crisis. The American Society of Plant Physiologists has tried consistently to build for the future, as well as to take care of the present. Many individuals have contributed thought, service, and money in order that we might grow toward

independence. The purchase of life memberships, and patronage have aided much; in addition the Society has set up machinery which has a tendency to strengthen our hands, and to provide a part of the overhead which is unavoidable in our existence.

Earlier in the year a report was promised on the endowments of the Society, as of the close of the 1939–40 fiscal year. These funds are listed as to principal, and available funds, at face value as follows:

BARNES Life Membership Endowment Fund,	Principal,	$3000.00
	Available,	52.30
STEPHEN HALES Endowment Fund,	Principal,	2000.00
	Available,	144.93
Life Membership, and General Endowment,	Principal,	3723.81
Total Endowments,		$8921.04

In addition to these funds there is a reserve, containing securities of unknown value. These have a face value of $1000.00, but represent reorganization properties, and cannot be given any definite value at present. If these were sound bonds, the endowments would be approaching a value of $10,000.00, obtained over a period of 15 years. May we not hope that an increasing number of our members may be challenged by these figures into joining with those who have given generously in the past? Gifts of any size are welcome; and one may become a life member or a patron at any time. There are seven patrons; there should be several times this number. And no better investment can be made by a young man than a life membership. In 20 years one pays the same amount in dues, and there is no gain to the Society's permanent funds. In 40 years, from age 25 to 65, the dues are double a life membership; and in addition to 100 per cent. interest to the individual, the Society finally has an increase in its permanent funds. There is also some satisfaction in feeling that one has contributed toward the permanent welfare of science. The treasurer will appreciate any contribution that may be made toward the building of permanency for the American Society of Plant Physiologists.

Errata.—A few errors in volume 14 of PLANT PHYSIOLOGY have been reported by authors. We appreciate the cooperation of those whose papers appear in the journal, and urge all to proofread and send in reports of errors observed. Please note the following:

Page 311, formula, for "Vg," read V_g, and for "a" read α.
Page 643, line 5 from bottom, for "weks" read weeks.
Page 645, citation 1, for "1037" read 1937.
Page 688, line 16, for "figure 3, curve d" read figure 2, curve D.

Hormone Information.—A mimeographed report, comparing the suitability of United States grown oats for the Avena test, has been prepared

by J. VAN OVERBEEK, California Institute of Technology, Pasadena, California. All Experiment Stations engaged in oat breeding were requested to send samples of oats for comparative tests. Victory oat, and Markton, proved to be most valuable for the auxin determination. Nearly 40 tests are reported, arranged in descending order of sensitivity. This valuable report may be obtained from the California Institute of Technology on request. It will aid much in the standardization of technique of making quantitative auxin measurements. Address requests to Dr. VAN OVERBEEK.

Naturalist's Directory.—The 32nd biennial edition of The Naturalist's Directory will be published in September, 1940. This directory contains the names, addresses, and special subjects of interest of naturalists in all parts of North and South America, as well as a list of periodicals and Natural History museums. The price of the directory is $2.50, and it is published by The Naturalist's Directory, Salem, Massachusetts.

Plantation Crops.—Technical Communication no. 13, of the Imperial Bureau of Horticulture and Plantation Crops, East Malling Research Station, near Maidstone, Kent, England, bears the title *Vegetative Propagation of Tropical and Subtropical Plantation Crops.* It has been compiled by G. ST. CLAIR FEILDEN and R. J. GARNER. It deals with the vegetative propagation of some 55 plantation crops. Major crops, such as rubber, coffee, cacao, etc., have received the attention of technical experts, and the foreign literature has been thoroughly combed for details of propagation of less familiar crops. One most valuable feature is the section devoted to methods used in vegetative propagation. The descriptions are supported by simple, clear, line drawings of some 17 types of graft, and 7 types of budding commonly used in vegetative propagation. Tropical workers will be interested in the illustrated account of the construction of loosely woven potting baskets which have been found useful as a substitute for pots in tropical nursery work. References to the original literature immediately follows the discussion of the propagation of each particular crop.

This valuable bulletin is priced at 3 shillings 6 pence. Orders may be addressed to the Imperial Bureau, East Malling, Kent, England.

French–English Dictionary.—A year ago we had the privilege of announcing a dictionary of German scientific terms by LOUIS DEVRIES, of the Iowa State College. We now are happy to announce that a companion volume, *French-English Science Dictionary*, by the same author, has just been published by the McGraw-Hill Book Co. It is an excellent volume for students and professors who have to read English scientific literature, or who must prepare for the modern language requirements associated with higher degrees. It makes no pretenses to completeness, but the vocabulary

is well-chosen, and covers the ground in a most satisfactory way. If students in reading meet words which they cannot find in the dictionary, the author requests that such words be sent to him for possible inclusion in future editions. The author and publishers deserve great commendation for the service they have rendered. The book should receive a wide and enthusiastic reception. It is an excellent desk companion, and its possession will certainly encourage more general reading of French scientific literature. The list price is $3.50.

Botany.—The third edition of HOLMAN and ROBBINS, *Elements of Botany*, has been published by John Wiley and Sons, New York. The main changes concern the recent advances in plant physiology, growth substances, culture solutions, erosion physiology, photoperiodic control of blooming, artificial pollination, self sterility, efficiency of photosynthesis, disease and weed control, economic value of plant products, and induction of chromosome changes. The quoted price is $2.75 per copy.

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